Notes on Sporozoa. Nos. II, III, and IV.[1]

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With Plates 9 and 10.

CONTENTS.

Preface.

While working at Rovigno during April and May, 1909, I examined a few of the common wall-lizards (Lacerta muralis), which occurred abundantly on the neighbouring islet of Figarola, as in the time when Prowazek studied the intestinal flagellates of this reptile (**26**). My object was really to see if a Trypanosome also occurred in it, Prowazek not having stated whether he examined the blood for that purpose or not; but I did not succeed in finding any Trypanosomes. In the blood-smears made from two individuals, however, a Hæmogregarine was found to be fairly plentiful. This Hæmogregarine is the same as that first described by Danilewsky (**6**) under the name Hæmogregarina lacertæ, and again later by Labbé (**13**), who placed it in a distinct genus, Karyolysus. I was too much occupied with other

[1] For the first of these Notes ("On Klossiella muris, Smith and Johnson"), vide 'Quart. Journ. Micr. Sci.,' vol. 48, 1904, p. 153.

work at the time to undertake a study of this parasite, so that I only made a few smears and cover-slip preparations from the blood.

Having an opportunity recently, I thought it might prove worth while to give some attention to these preparations, particularly to those stained by iron-hæmatoxylin, because—so far as I am aware—no observations have been made up to the present upon the nuclear structure of Karyolysus, as it is seen when the parasite is fixed and stained by the best cytological methods. My idea was, principally, to compare the nucleus of this Hæmogregarine with that of the piscine form (Hæmogregarina rovignensis) from Trigla lineata, an account of which has been given by Minchin and Woodcock (**20**). I had not long examined my preparations, however, before observing that a remarkable agreement was apparent between the nuclear condition of Karyolysus at a certain period of the life-cycle and that of a particular Coccidian in the corresponding phase. A study of the different forms of individual present in my smears has led me to the conclusion that they all belong to one species of parasite. This result has an important bearing, in my opinion, upon the question of the distinctness of many of the so-called species of Lacertilian Hæmogregarine which have been described, as I hope to show below. Lastly, the observation of the occurrence of a prominent karyosome, whose behaviour agrees closely with that of the characteristic coccidian karyosome, induced me to study again, from this point of view, the nuclear condition present in Leucocytozoon and Halteridium, as it is found in these parasites when fixed and stained in a similar manner.

II. Observations on Karyolysus lacertæ (Danil.), together with Remarks upon the Specificity of the Hæmogregarines of Lizards.

I will first give an account of Karyolysus as it occurs in my preparations. With two or three exceptions, all the

individuals observed are intra-cellular. In the blood of both the infected lizards the great majority of the parasites occur under one of two different aspects, which might lead one, at first sight, to conclude that two distinct species were concerned; but after a careful comparison of many individuals of both kinds, no doubt is left in my mind that they represent respectively early young phases and rather later, older forms of one and the same parasite. The two types of form (as they may be designated for the present) are distinguished chiefly by the position and character of the nucleus, which I will consider in detail presently; the latter feature can only be studied properly in preparations made by the "wet" method. I will merely say here that in the first type of individual the nucleus is situated more or less about the middle of the body (Pl. 9, figs. 2–8, 19–29), whereas in the other type it is close to one end (figs. 9–18, 30–40).

As regards their general appearance, both kinds of individual are usually bean-like in shape, the younger parasites being more slender and now and then slightly crescentic, the older ones broader and stouter. The individuals of both types vary somewhat in size, the former being, as might be expected, slightly shorter on the whole and distinctly narrower than the latter; but a few forms which possess the nuclear characters of the first type are met with, which are approximately as large as others possessing a nuclear arrangement of the second type. The dimensions of the younger forms, as seen on "wet" films, vary from 8 μ by $2\frac{1}{8}$ μ (fig. 22) to $9\frac{1}{2}$ μ by $2\frac{1}{2}$ μ (fig. 26); those of the older individuals from 9 μ by $2\frac{1}{4}$ μ (fig. 39) up to $11\frac{1}{2}$ μ by 3 μ (fig. 33). On "dry" smears the larger parasites are probably rather flattened out; the extreme limits of variation in size (of either kind of individual) noticed are from 11 μ by $2\frac{1}{2}$ μ (fig. 2) up to 13 μ by $4\frac{1}{2}$ μ (fig. 17). The largest bean-shaped individuals, however, such as those of figs. 14–17, have undoubtedly acquired that appearance secondarily, by the lateral fusion of the two arms of a U-shaped form, the U-shaped form resulting in the first place from the further

growth and extension of the body-cytoplasm of a smaller individual. I have found different stages of the process in my preparations. The development of the U-form takes place only, so far as I have observed, in those parasites in which the nuclear position is that of the second type mentioned above. It begins by the formation of a small outgrowth at one end of the body, which is at once curved back and so extends backwards close along one side of the body (figs. 10, 36, 40). This outgrowth may arise either at the nuclear end of the parasite or at the opposite one, more usually, I think, at the latter. As it grows this process gradually forms one arm of the U, and at length the two arms become more or less equal (figs. 14, 15). Ultimately the two arms unite and a stout bean-shaped form results.

In nearly all of the individuals observed in "wet" preparations, of whichever type they may be, immediately surrounding the body of the parasite is a distinct space, which in some cases is very marked (cf. figs. 20, 22, 24–40). This space is probably due to the greater contraction of the parasite, as a result of the technique, than of the cytoplasm of the red blood-corpuscle enclosing it, thus causing a shrinkage of the former away from the latter. In ordinary "dry" smears, stained with Giemsa, this space is also often seen, though not so regularly as in the other preparations (cf. figs. 5–8, 12). In the case of the smaller parasites there is probably no definite membrane or envelope bordering the space on its outer side, distinct, that is to say, from the inner margin of the cytoplasm of the corpuscle (cf. for instance fig. 19, where the young Hæmogregarine has obviously just entered the host-cell). In the older (larger) forms, however, there is certainly a definite envelope present, constituting a delicate but firm capsule around the parasite (cf. especially figs. 37–40). In the case of two of the parasites figured it will be noticed there is no sign whatever of the cytoplasm of the blood-corpuscle; the reason for this will be mentioned shortly (pp. 177, 179). Hence the capsule surrounding the parasite is very conspicuous. In many cases where the cytoplasm of the

host-cell is still apparent the delicate capsule does not stand out in such a marked manner, but its presence is clearly indicated, in my opinion, by the following consideration. Many of the older forms, those, i.e., of the second type, in which the nucleus is situated near one end, appear very dark in "wet" preparations, being stained diffusely and more or less uniformly, so that it is very difficult to distinguish the nucleus. This appearance is really owing to the stain deposited inside the capsule or envelope not having been sufficiently extracted subsequently, the differentiating agent not having had time to penetrate properly inside the capsule, thus leaving the parasite overloaded with stain. I have never found this state of things, it should be noted, in the younger parasites, with the nucleus still near the middle; hence the capsule does not appear to be formed during the early phase. This capsule or envelope present in certain forms of Karolysus appears to be very similar to that described in the case of small forms (young schizonts) of Hæmogregarina triglæ by Minchin and Woodcock (20). In the case of Karyolysus, however, I am inclined to think that the capsule is rather a definite envelope formed by the parasite than merely a sheath or altered layer of the cytoplasm of the blood-corpuscle (cytocyst), as we regarded it at the time in H. triglæ; its persistence and distinctness in such individuals as those drawn in figs. 37 and 38 supports this view.[1]

Considering now the nuclear structure in detail (as it is seen in "wet" preparations, stained with iron-hæmatoxylin), in the first type of individual, where the nucleus is situated near the centre of the parasite, the most striking feature is the very frequent occurrence of a conspicuous, deeply staining body, which is closely associated with the nucleus, lying at one side of it, contiguous to, but not actually forming part of, the general nuclear substance (figs. 19, 21–25). This latter consists, as in other Hæmogregarines, of a network containing small but fairly prominent grains of chromatin, most of which

[1] The mode of origin of the capsule may be really the same, of course, in H. triglæ also.

are usually disposed near the periphery. The limit or border of the nucleus is well-defined, but I am a little doubtful whether it can be regarded as constituting a true nuclear membrane. In some cases there are two of the above-mentioned conspicuous bodies, approximately at opposite sides of the nucleus (figs. 20, 26); these are generally unequal in size, neither being as a rule so large as when there is only one. Frequently these elements are seen to be surrounded by a very clear zone or halo (figs. 19, 20 and 25).

In the other type of individual there is usually no such large, deeply staining element associated with the nucleus (figs. 30, 32–36, 38–40). This contains fairly uniform grains of chromatin, which, on the whole, are distinctly more prominent and stain rather more deeply than those in the nucleus of the first type; now and again one of these grains is seen to be somewhat larger than the rest. Nevertheless, in a few instances, parasites belonging to this second type of form, with the nucleus near one extremity, do also show a large, deep-staining body in close association with the nucleus (figs. 31, 37), which is quite comparable to, or at least represents, that seen in the case of individuals of the other type. Much more frequently, however (though not always), in place of this element close to the nucleus there is noticeable a body lying at or near the surface of the protoplasm of the parasite, usually about the middle of its length (figs. 32–36). This structure may be nearly as large as that just described, but it is generally smaller, and may be very inconspicuous (fig. 36); where it is large it stains fairly intensely, but it is never so dark and black-looking as in the other cases, and, moreover, it has a much duller appearance and not such a well-defined outline.

I propose to leave for the moment the question of the significance of these bodies. It may be added that in Giemsa-stained smears on the other hand, in which the nucleus of the parasites generally appears to consist of large, irregular or ill defined masses of chromatic substance, it is only seldom possible to distinguish a more deeply staining element at

one side, which probably represents the characteristic element above described (cf. fig. 8).

The effects of the parasite on the host-cell are very pronounced and characteristic, as is well known to be the case, of course, in Karyolysus. The gradual alteration of the red blood-corpuscle and its appearance when infected by the different forms of parasite merit description, however, since this change is of great importance and assistance both in determining the relation to each other of the two chief types I have described, and also in connection with the question of the various species of Hæmogregarine (Karyolysus) said to occur in this lizard. The earliest change in the appearance of the host-cell which I have noticed is drawn in fig. 2 (from a Giemsa smear). The parasite infecting this corpuscle is one of the smallest observed, and has the nucleus centrally placed. Comparing the host-cell in this case with an ordinary uninfected red blood-corpuscle, its nucleus is found to be already distinctly larger, i.e. hypertrophied, but still oval in shape and not much elongated. The cytoplasm of the corpuscle is also slightly hypertrophied, but it is still stained to about the same degree and shade of colour as in an uninfected cell. This is, however, almost the only instance I have noticed where the cytoplasm appears stained similarly to what is the case in an uninfected cell. It is remarkable how quickly the presence of a Karyolysus-individual in a corpuscle produces some effect on the cytoplasm which results in a complete alteration of its staining properties. In nearly all the corpuscles infected with this Hæmogregarine, the cytoplasm has either taken up the stain only slightly, being faintly coloured, or else is very pale, practically unstained, so that it is often a matter of extreme difficulty to discern it at all. This is especially the case in wet preparations, stained by iron-hæmatoxylin; and in this respect Karyolysus differs markedly from certain other intra-cellular parasites of red blood-cells, of which I have preparations stained in a similar manner. For example, in Hæmogregarina triglæ (cf.

the figures of Minchin and Woodcock, loc. cit.) and again in Halteridium noctuæ (cf. below), the cytoplasm of an infected corpuscle is usually stained deeply, like that of an uninfected one, even where, as in the former case, there is a certain amount of hypertrophy; in these, the parasite appears as a clear space, almost vacuole-like, surrounded by the dark cytoplasm. In Karyolysus the appearance is quite different. Figs. 20–22, 24–26 represent early stages in an infection as early as, or slightly later, than that of fig. 2, from a Giemsa smear. The nucleus of the corpuscle is either oval or beginning to elongate. In such cases the cytoplasm can still be made out, but it never appears any darker than is indicated in Figs. 20, 22, 24. The nucleus also retains the stain much less intensely than in an uninfected cell stained by the same method (cf. fig. 23), the actual masses and grains of chromatin standing out sharply from the finely granular or reticular ground substance. The host-cell nucleus, it will be seen, is at once displaced by the parasite, and pushed to one of the longer sides of the corpuscle.

From being oval or slightly extended, the host-cell nucleus gradually becomes considerably elongated and greatly narrowed, i. e. compressed (figs. 32–40); all stages in this transition can be found, the real change in shape being best realised, of course, in preparations stained by iron-hæmatoxylin. In most cases the corpuscle-nucleus, in its final condition, appears like a slightly crescentic band, which is closely apposed to the parasite (or rather to its envelope) and follows its contour, curving round somewhat at either or both ends; this portion of the cell-nucleus is generally a little broader, i. e. less compressed than the rest, giving the whole nucleus the appearance of a bent club or halter, as the case may be.[1] In all these instances the axis of extension of the host-cell nucleus is approximately parallel to the length of the parasite. Now and again, however, where the corpuscle-

[1] The resemblance between this hypertrophied nucleus and that of the spindle-shaped host-cell infected by Leucocytozoon is often striking (cf. figs. 11, 12, 18 and 19, Pl. 10).

nucleus has either not been quite parallel to the longer axis of the cell to start with, or else has become twisted round somewhat by the entry of the parasite, the longer axis of the Karyolysus is more or less oblique to that of the host-cell nucleus, the one lying, as it were, across the other; in these cases, fission of the host-cell nucleus into two or more portions nearly always results (figs. 29, 11, 30). An important point must be mentioned regarding the appearance which one of these nuclei, in its final condition of hypertrophy, may occasionally present on a Giemsa smear, since it affords, I consider, another example of how the over-staining tendency of this stain may mislead and cause erroneous interpretations. In a few cases a mass of staining substance is seen, fitting like a cap round one end of the parasite, or there may be such a mass round both ends (figs. 16–18). These masses stain similarly to the nucleus of the host-cell, lying at one side of the parasite, and in fact may be distinctly connected with this and manifestly portions of it; it may happen, however, that such a mass appears almost or entirely separate from the nucleus, especially in flattened-out parts of the smear. Nevertheless there can be no doubt that these caps of staining substance represent also in such cases merely the wider, club-shaped end-portions of a crescentic host-cell nucleus, as above described, only here they are greatly overloaded with stain. These "caps," it is important to note, are distinctly on the outer side of the capsule enveloping the Hæmogregarine.

As indicated above, the cytoplasm of the infected corpuscle becomes ultimately so colourless that it is quite impossible to discern it (cf. figs. 37, 38 from wet preparations and fig. 17 from a Giemsa smear); in these cases it cannot be said whether it is still present or not.

The two forms of the parasite can now be considered in relation to the particular degree of alteration shown by the host-cells respectively infected by them. As a rule, in corpuscles which are in the earlier stages of alteration, with the nucleus still oval or only beginning to elongate, the parasites are of the first type described, with the nucleus

central and having the conspicuous, deeply staining body in close association with it (figs. 20–26). On the other hand parasites of the second type, with the nucleus near one end of the body, occur nearly always in corpuscles in which the alteration is far advanced, the cytoplasm being, at the best, only with difficulty discernible and the nucleus greatly elongated and narrowed (figs. 32–40). As is only to be expected, however, occasional exceptions to the above regular conditions are to be met with. Thus, an individual may be found, having its nuclear arrangement of the first type, which has already caused considerable elongation and alteration of the host-cell nucleus (figs. 8, 28, 29); conversely, a parasite may have acquired the second type of nuclear condition before the corpuscle-nucleus has become very elongated and narrow (figs. 10, 31). It may be regarded as practically certain, therefore, that the second type of individual is a rather later or older phase in the development of the first type of parasite. In addition to the evidence afforded by the various stages in the alteration of the infected corpuscles, this conclusion is also supported by the following points. Parasites of the second type are on the whole distinctly larger, that is to say, they have more bulk than those belonging to the first category; further, the only individuals seen free (fig. 21), or which have manifestly only recently entered a corpuscle (figs. 19, 26), have the first type of nuclear arrangement.[1]

It is not difficult, I think, from a careful comparison of different individuals, to form a fairly accurate idea of the manner in which the change in nuclear position and character is brought about; and for this purpose it is necessary to study the behaviour, in relation to the nucleus, of the characteristic deep-staining body which is associated with the latter in the young forms of the Hæmogregarine. In the earliest phase this body, which from now onwards I will designate according

[1] I have never observed any individuals of the larger, older type free—that is to say, which could have been liberated from a corpuscle, whether with or without the enveloping capsule.

to its true significance, namely, as a karyosome, is single and relatively large; it is situated at one side of the general nuclear substance, apparently extra-nuclear. This large karyosome next undergoes unequal division. The process takes place in a particular manner, which is neither amitotic nor yet a well-defined mitosis. This method of division has been usefully distinguished by Nägler (22) as "promitosis."

It may be as well to indicate, first of all, what is meant by promitosis. Its characteristic feature is that the division is initiated and carried out by means of an internal division-centre, which itself first divides, the two resulting daughter-centres then passing away from each other, but remaining connected by a distinct fibril or axial thread, the "centrodesmose." The term "promitosis" was originally applied by Nägler to nuclear division taking place in this manner, the intra-nuclear division-centre being a "nucleo-centrosome" or a karyosome. Where the karyosome plays this part, however, the true division-centre—certainly in most cases, and perhaps always—is an intra-karyosomatic centrosome or centriole, which initiates the process, although, owing to the intensity with which the karyosome usually stains, the centriole itself can rarely be distinguished, its presence being often only actually discernible at some other period in the development (cf. below, p. 182). Fortunately, however, the axial fibril or centrodesmose connecting the two separated daughter-centrioles persists often for a long time, even after the division of the karyosomatic or nuclear material is completed; hence it is just this stage of the division-process which is most likely to be observed. Therefore, where two nuclei (or karyosomes) are seen still connected by a definite centrodesmose, it may be safely concluded that the division has been brought about by an internal division-centre (centriole), in a promitotic manner. It only remains to say that I consider the term "promitotic division" can also be applied very suitably to the division of a karyosome, where this occurs unaccompanied by, or independently of, the division of the nucleus itself; Jollos (12) has already used the term in this connection.

It is undoubtedly in the above-described promitotic manner that the unequal division of the karyosome takes place in the young forms of Karyolysus; for I have found two or three examples which show very clearly the still persistent centrodesmose between the two halves (fig. 20). This fibril stretches apparently across the general nuclear mass; but it may really lie outside it, i. e. above or below; I do not feel sure upon the point. The smaller daughter-karyosome resulting from the division always comes to lie at the opposite side of the nucleus to the other, larger one (figs. 24, 26). This smaller, secondary karyosome, however, soon becomes incorporated with the general nuclear material; either it is distinguishable as a rather larger and more prominent grain, or else, probably having undergone further subdivision, it can be no longer distinguished from the rest of the chromatic substance. Now and again, it may be mentioned, in such a nucleus a small, but sharp and well-defined granule is seen in the centre; this may very likely be the centriole (fig. 40). The nucleus has by this time changed its position and passed to one end of the body of the parasite. In the majority, if not in most cases, it leaves behind it the larger half of the karyosome, which resulted, i. e., from the original promitotic division; this remains near the middle of the body, the nucleus simply moving away from it. Why this change in the nuclear position occurs I cannot say; it might be supposed, perhaps, that it had some connection with the commencing development of the U-form of the parasite, but the bending of the cytoplasm sometimes takes place at the end opposite to that to which the nucleus travels. Whatever the reason, this movement occurs, I should say, very rapidly, for I have not succeeded in finding an individual which shows the nucleus caught in the act, as it were, halfway between the end of the body and the stationary karyosome. This latter element thus left behind takes no further share in the nuclear development, and appears to be entirely discarded. As already indicated, it alters considerably in staining properties and in definiteness of outline; it gradually becomes

smaller and smaller, being perhaps partially used up by the cytoplasm, and ultimately its remains are seen at the surface of the body (figs. 34, 35). Often, however, no trace of this body is left (figs. 30, 38 and 39). On the other hand, occasionally this large karyosome seems to persist and to change its position with the nucleus (figs. 31, 37). In such cases it lies nearest to the end of the body, between this and the nucleus, having been pushed along as it were by the nucleus, instead of being left behind. Possibly the reason for this occasional persistence of the large karyosome as a separate element in close association with the nucleus, after the latter has changed its position, may be that the karyosome has not yet undergone the above-described division—a division which may be necessary in order to eliminate an unrequired portion of the karyosomatic material before the remainder is added to the nuclear substance. I have no evidence as to the further behaviour of the karyosome in these cases.

I can now summarise the general course of the early development in Karyolysus, so far as I was able to ascertain it. The different types of form observed are phases of one parasite. A small individual, such as that of fig. 21, penetrates a red blood-corpuscle (fig. 19) and begins to grow. As the parasite grows, changes in the nuclear constitution and position take place. At about the same time a definite envelope or capsule is formed around the parasite, inside which the latter tends to acquire, by bending up, a characteristic U-shape, and ultimately becomes stout and bean-like. The presence of the Hæmogregarine causes very great changes in the appearance of the host-cell, hypertrophy and pronounced alteration in the shape of the nucleus, sometimes its fission; further, the cytoplasm, or what remains of it, loses almost entirely its staining properties and becomes extremely difficult to see in the preparations.

From a comparison with Reichenow's valuable and detailed account (27) of the development of Hæmogregarina stepanovi of the tortoise, there can be little doubt that

the forms I have described of Karyolysus are phases in the development of the schizont, i. e. the form which undergoes schizogony or endogenous multiplication. A point in regard to which I cannot be certain is whether these young schizonts are the first to be developed, as the result of a fresh infection, or whether the infection is of some standing and these forms have been produced by a prior schizogony; in other words, whether the small, free individuals are developing sporozoites or merozoites. The only indication bearing upon the point which I can note is that the nuclear constitution of the young individuals, showing a distinct excentric karyosome, agrees markedly with the nuclear condition found in the developing merozoites of certain Coccidia and differs from that present in the sporozoites. I intend to discuss this agreement more particularly later, and will merely say here that this evidence favours the view that the schizonts which we have been considering are developing from merozoites.

The Question of the Specificity of the Hæmogregarines of Lizards.

I wish now to discuss the question of the specificity or true distinctness of certain of the many alleged species of Hæmogregarine (Karyolysus) which have been described from Lacerta spp., chiefly from the common European species agilis, muralis and viridis; my object is to show that some, at any rate, of these new species are almost certainly nothing more than different forms or phases of one and the same parasite, Karyolysus lacertæ. As I have had occasion to point out more than once in previous papers, the custom is far too prevalent of regarding any difference in appearance, or variation in size or form, observed in individuals of a certain genus of blood-parasites (and particularly in the case of Trypanosomes and Hæmogregarines), as indicating a distinct species, even though this "new species" occurs in a host in which a parasite of the same kind is already known. Often the view which is at least quite as probable, and in many instances more so, namely that the

forms in question are phases in the life-history of one and the same species of parasite, receives no consideration, and no attempt is made to connect the various types by means of intermediate stages. I am glad to see that Laveran and Pettit, in a recent note (**15**), also express a similar opinion, and comment upon the confusion liable to be caused by creating new species in the above casual manner.

To begin with the original description of Hæmogregarines from lizards, i. e. the account given by Danilewsky (**6**), this author observed various forms of the parasites in L. agilis and viridis. Making all allowance for the fact that Danilewsky's description and figures are mostly based on observations on the living parasites in the drawn blood,[1] and also for the primitive character of microscopical technique in those days, it seems probable nevertheless that this author was actually dealing with more than one species. Here, as in other cases (for instance, his memoirs on Trypanosomes), it is extremely difficult to gather what Danilewsky intended to mean by his grouping of different forms and the nomenclature he applied to them. He distinguishes three intra-cellular types (A, B and C), which he regards as having a genetic connection ("lien génétique") with one another. To these, collectively, he gives the name Hæmogregarina lacertæ; but immediately afterwards the second type (B) is termed Drepanidium lacertarum, because it is smaller and younger; while in another part of the memoir the third form (C) is called Hæmocytozoon clavatum! The last type is generally considered to be distinct; this is, I think, most likely, particularly since it does not produce, to judge from Danilewsky's account, hypertrophy of the blood-corpuscle and alteration of its nucleus; in other words, it is apparently

[1] While, of course, for many points, e. g. behaviour, movement, living observations are invaluable, it cannot be pretended that such can be relied upon where comparative questions of size, form and minute structure are concerned, especially in the case of intra-cellular blood-parasites, which, as is well known, frequently alter or else become deformed in drawn blood.

not a karyolysing form at all. The small type (B) may be a young phase of (A); more than this cannot be said. At any rate it is to the first described parasite (type A) that the specific name lacertæ really belongs. Comparing the different forms of the Hæmogregarine I have described above, from L. muralis, with Danilewsky's description and figures of H. lacertæ, it is perfectly clear that the parasite is the same species in both cases, and, moreover, in the same period of development; some of Danilewsky's figures are of young forms, with the nucleus near the middle and the host-cell only slightly altered; others are of the older phase, with the nucleus at one end and the nucleus of the corpuscle completely karyolysed.

The next account of Hæmogregarines from lizards was that of Labbé (13), who described parasites of this nature from L. muralis, viridis and ocellata. Labbé considered that the various forms which he observed belonged to two distinct genera, to which he gave the names Karyolysus and Danilevskya respectively. With the series of forms comprised in the latter genus we are not here concerned; it is very doubtful whether any are included which should really be kept separate from the ordinary genus Hæmogregarina.[1] In the genus Karyolysus

[1] It may be noted, however, that Labbé seems to have paid no regard at all to the laws and standards of nomenclature, for he deliberately placed in this genus the parasite of Cistudo europæa, originally described by Danilewsky under the name Hæmogregarina stepanovi, that is to say, the type-species of the genus Hæmogregarina in other words, at his own pleasure, he replaced the generic name Hæmogregarina by that of Danilevskya. If he wished thus to commemorate the Russian savant's name he ought, of course, to have called the parasite which he distinguished as Karyolysus by his name instead. Moreover, for the species of "Danilevskya" which he found in lizards he created the name lacazei, although saying at the time that this was probably the same form as that distinguished by Danilewsky as Hæmocytozoon clavatum. In any case, therefore, this Hæmogregarine of lizards should bear the specific name clavatum (not lacazei), and if it does not belong to the genus Hæmogregarina, the generic name Hæmocytozoon, not Danilevskya, must be given to it.

Labbé placed forms which he regarded as similar to those described first by Danilewsky under the designation H. lacertæ. Why, in so doing, he altered the specific name to lacertarum it is difficult to understand; the name should read, of course, K. lacertæ (Danil.). From a study of Labbé's description I do not think there is any reason to doubt that this author was dealing, in the main, with Danilewsky's parasite, H. lacertæ; though it is true that certain of his figures may represent some other Hæmogregarine. Unfortunately, Labbé does not give any details about the particular species of lizard in which the various types of the parasite he figures respectively occurred. Since he examined four different species of host, in certain of which, at any rate, another Hæmogregarine is also parasitic (as, indeed, he recognised, distinguishing this latter by the name "Danilevskya" lacazei, see footnote, p. 186), it is quite possible that he did not altogether succeed in separating the two forms. Nevertheless, leaving out of consideration his description of the "endoglobular sporulation,"[1] Labbé's account of the appearance, size and structure of the young and adult parasites in the blood-corpuscles, and in particular his description of the marked alterations in the host-cell, make it perfectly evident that most of his observations did actually refer to the same parasite as that described by Danilewsky, and as that which I found in the lizards I examined.

In 1901, Marceau gave an account (18) of the Hæmogregarine parasites which he observed in L. muralis, and in this lizard alone; and here also it is quite obvious that the author was dealing chiefly, if not entirely, with K. lacertæ. On the whole, Marceau's description agrees closely with that of Labbé.

It is sufficiently clear, I think, that there is a definite

[1] This process doubtless represents the schizogony of the parasite, which is apparently either of a double character, similar to that described by Reichenow (27) in the case of H. stepanovi, or else of a type where sexual differentiation is already manifest.

parasite, occurring in L. muralis and probably also in L. agilis and viridis, for which the specific name lacertæ must be retained. Further, in my opinion, it is also preferable to retain Labbé's distinct generic name Karyolysus for this Hæmogregarine, as also for any other similar form which may produce the same characteristic effects upon the host-cell; I certainly consider such forms can be advantageously grouped together—if not in a separate genus, at any rate in a distinct sub-genus—on account of their peculiar behaviour in this respect. It is only necessary to compare the effect on its host-cell produced by an ordinary Hæmogregarina to realise that there is a marked difference between the two types of parasite. Species of the genus Hæmogregarina, whether from fishes or repitles, may often cause more or less hypertrophy of the red blood-corpuscle; but they never stimulate, as it were, the cell-nucleus to undergo such profound changes as is the case with Karyolysus, where the nuclear alteration begins, as I have shown above, almost as soon as the parasite has invaded the corpuscle. I need only refer, by way of illustration, to the recent figures published by Minchin and myself (loc. cit.) of H. triglæ, by Neumann (**23**) of various piscine Hæmogregarines, by Reichenow (loc. cit.) and also Hahn (**9**) of H. stepanovi, and lastly, the figures of many species from snakes given by Sambon and Seligmann (**29**)[1]. In all these cases the host-cell nucleus is practically unaltered; it may be now and then slightly flattened in appearance, but this is usually where it has been pushed to one side of the cell by the growing parasite, and is obviously due to a mechanical cause. It may be said, of course, that if a separate genus Karyolysus is to be thus recognised, the distinction between it and Hæmogregarina will be based mainly, if not entirely, on biological grounds. This is, no doubt, true; but one has not to look far for other instances where a generic distinction, which is generally accepted, is recognised for biological

[1] Some of these last should clearly be placed in the genus Karyolysus.

reasons, which, if not just the same, are of a similar order. Thus the avian blood-parasite known as Leucocytozoon is distinguished from that known as Halteridium, although there is little doubt that the two types are very similar in structure and in regard to the essential features of the life-history; the principle difference is that of habitat, the one form (Halteridium) being parasitic in the red corpuscles, the other (Leucocytozoon) in the uninuclear leucocytes.[1] Nevertheless, it is very useful to continue to distinguish the two types as separate genera. And similarly as regards these reptilian blood-parasites, as a means of indicating at once the characteristic difference in the effects on the host-cell, it is most convenient to retain the generic names Karyolysus and Hæmogregarina for the karyolysing and non-karyolysing group of species respectively.

Of late years several workers have given accounts of Hæmogregarines from lizards, for the most part recording the occurrence of new parasites—or at any rate, parasites regarded as new—in various additional hosts; several of these are undoubtedly Karyolysus-forms. The parasites of the different species of Lacerta have been studied chiefly by Laveran and Pettit and by Franca. In their first paper, Laveran and Pettit (**14**) describe the parasites they observed in L. muralis and viridis, more frequently in the former species. They distinguish three different types, all of which they consider to represent Danilewsky's parasite, which they term H. lacertæ; the authors thus use the correct specific name, but prefer to keep the parasite in the genus Hæmogregarina. The first two types are the same as those which I have again found, that is to say, young schizonts and older ones. The only point which requires notice is that the authors consider there is no capsule, but merely a shrinkage space around the second type of form; this is certainly a mistaken view on their part. The third form of parasite is, in my

[1] The different habitat explains, of course, the fact that the one parasite (Halteridium) produces melanin pigment, while the other Leucocytozoon) does not.

opinion, a type rather different from any phase so far described by other workers, and from anything I have observed. It is a large, curved form, certainly a Karyolysus, because of its effect on the cell-nucleus; I should say it probably represents another phase of K. lacertæ, but until the life-cycle is better known or until this form has been connected by intermediate stages with other known phases, the matter must remain uncertain.

França, in a series of papers on the Hæmogregarines of lizards, chiefly species of Lacerta, has been unfortunately preoccupied with the idea that almost every variety in form and appearance of parasite observed represents a distinct and independent type, with the result that he has greatly complicated and confused the subject of these Hæmogregarines of lizards. Thus, in more than one case, the author creates several new species for parasites from the same host, in some instances basing the distinctions between them on such slender grounds as the different staining appearances (tint of colour, presence or absence of granulations, etc.) exhibited. Now, França's figures are all from preparations stained by some modification of the Romanowsky method; and, as is well known, the great variability and uncertainness in the staining appearance presented often by the same object at different times, even where the smear has been treated, so far as was known, in exactly the same manner, renders it perfectly useless to label as distinct species forms showing differences in appearance after being stained by a Romanowsky method, mainly or solely on this ground. Again, França is of the opinion that it is unlikely that a particular species of host will be infected with the same species of parasite in different countries, or even in different districts of the same country. I can only say I do not share this view at all. We know, for example, that Trypanosoma lewisi occurs in rats all over the world; and other common parasites, e.g. certain Gregarines and Coccidia, are known from the same species of host in various countries. I do not think there is any reason to doubt that the same species of Hæmogregarine may occur in the L.

muralis of Portugal, for example, which is found in that lizard in Russia, and again in Southern Austria, and in France. I associate myself entirely with the remarks of Laveran and Pettit in their later note (15) with regard to this matter.

In one of his memoirs (8), França describes the different forms of hæmogregarine which he found in L. muralis in Portugal. The author leaves out of account altogether the species K. (H.) lacertæ. This he does for two reasons: firstly, in accordance with the view just referred to, because of the different geographical locality of the host in the case of the lizards which he examined; and secondly on the ground that several different forms have been really included in the specific designation lacertæ. From what I have shown above, it will be evident that, on the contrary, we can recognise and clearly distinguish a well-defined species, to which the name lacertæ belongs by right.

França creates no fewer than four new species, all from this one host, namely, H. nobrei, bicapsulata, marceani and nana. These different parasites usually occur associated together in various groupings; and it is the exception rather than the rule to find them separately. The first three are typical karyolysing forms, and hence may be termed Karyolysus. The last named, it should be pointed out, is, as its name implies, a very small form. From the only figure given it is obvious that this is merely a young phase; it cannot itself be regarded as an adult parasite, and in its older phases it may possibly be identical with one of the other types described. At any rate, it seems distinctly premature, in the circumstances, to give this type a new specific name. As regards França's other three species, I confess straightway that I consider they are only different forms or phases of our old friend K. lacertæ. I have come to this conclusion principally on two grounds; in the first place as a result of the detailed comparison I have myself made of certain forms of K. lacertæ, and of the alterations produced in the infected host-cells as seen in smears stained with Giemsa and also in wet preparations stained with iron-hæmatoxylin; and secondly,

as a result of the valuable light thrown on the whole subject of the life-cycle of a Hæmogregarine by Reichenow's work on H. stepanovi. Of course, this work has appeared since França's papers were published, so that we have now a guide to the interpretation of the various phases which was then unavailable. As the general scheme of the life-cycle, so far as it is undergone in the Vertebrate host, has been shown by Miss Robertson (28) to be fundamentally similar in the case of another Hæmogregarine also, I think we may regard it as probable that the life-cycle is similar, in its main traits, in other reptilian Hæmogregarines; and there is no need to consider that of Karyolysus as likely to be very different from that of Hæmogregarina merely because of the biological differences between the two forms, i.e. with respect to the behaviour and reaction of the host-cells. Assuming a general agreement, a particular type or stage of parasite observed in a lizard might represent any of the following phases in the life-cycle of a single species: The young growing schizonts produced from the sporozoites in a new infection; the merozoites or growing schizonts resulting from a first type of schizogony, e.g. with many merozoites (micromerozoites?); the merozoites or young schizonts resulting from a second type of schizogony, e.g. with few merozoites (macromerozoites?); lastly, the growing gametocytes, which may themselves be differentiated. As these various phases very likely show definite, though it may be slight distinctions from one another, if they were only observed casually, as it were, and their further development was not followed, nor their connection with one another ascertained, some would at once jump to the erroneous conclusion that they constituted distinct and new species.

Considering França's three Karyolysus-forms separately, K. (H.) bicapsulata, which we may take first, is so named because of two caps of deeply staining matter which occur one at each end of the parasite. From França's fig. 7 it is seen very clearly, in the first place, that these "caps" are distinctly outside the true envelope or capsule of the Hæmogregarine, and secondly, that they resemble closely in appear-

ance the hypertrophied nucleus of the host-cell, and, in fact, may be connected with the latter (for instance, the cap on the right-hand side of the upper parasite of fig. 7). Now, as stated above, I have observed a very similar appearance in some individuals of K. lacertæ in Giemsa-stained smears (cf. figs. 16–18). In my opinion there is no doubt whatever that these "caps," in the case of França's parasite also, are simply the result of the alteration to the nucleus, the thicker or club-shaped end-parts of which curve round the parasite and may be almost or quite detached from the middle portion; these caps have nothing whatever to do, directly, with the parasite. A perfectly similar behaviour of the nucleus of the blood-corpuscle has been described by Billet [2] in the case of K. (H.) curvirostris; two of this author's figures show exactly the same condition. Other points about França's account of K. "bicapsulata," e.g. the average size, the presence of a definite envelope around the parasite, make me practically certain in my own mind that this is not a new species at all, but only a phase of K. lacertæ corresponding to the second, older type described above. I should add, however, that Laveran and Pettit also seem to regard this "bicapsulata" as a distinct species, although they say that they found it associated with lacertæ, and mention further that, in deeply stained specimens, the "caps" stain very similarly to the deformed host-cell nucleus!

K. (H.) nobrei. This form Laveran and Pettit (loc. cit.) themselves consider resembles K. lacertæ so closely that it is doubtful whether it is really a distinct species. In my own preparations I have not come across any individuals which exactly represent this form; the parasite drawn in fig. 15, however, shows considerable resemblance in size and general appearance to the form figured by França in his fig. 2, the chief difference being in the position of the nucleus, which is near the middle of the parasite in França's case. I should say it is very likely that this is just one of those cases referred to above, where a different phase in the life-cycle of the parasite has come under observation. From a consideration

of França's figures relating to K. nobrei, the suggestion may perhaps be put forward that the phase in question corresponds to the second process of schizogony (with few merozoites) which occurs in H. stepanovi, and the type of individual immediately preceding or resulting from the same.

Again, with regard to K. marceaui, a form occurring in the blood is practically indistinguishable, according to certain of França's figures, from some individuals of the second type of K. lacertæ, which I have described; thus my figs. 9 and 12 agree closely with his figs. 9 and 10 respectively of K. marceaui. França also mentions and figures certain phases from the liver, which he considers represent conjugation. What these do exactly signify is uncertain, but the micro- and macrosporogony described as resulting from this process is quite comparable to Marceau's account of the same process in what is admittedly K. lacertæ. (It may be added that in both cases it is of course much more probable, considering the matter in the light of Reichenow's work, that schizogonic multiplication is concerned.) Hence, on the whole, and at any rate until the life-cycle of K. lacertæ has been thoroughly worked out, it is very much better, I think, not to adopt these new names, bicapsulata, nobrei and marceaui, which would only entail great confusion and difficulty, but to consider them as representing merely different phases of K. lacertæ.

To complete my summary of this question, I must mention that there has been the same premature and probably useless multiplication of species in the case of Karyolysus-forms from another species of Lacerta, viz. L. ocellata. In the first place, Billet [2] gave a short account, already referred to, of a karyolysing Hæmogregarine occurring in this lizard in Algeria, to which he gave the specific name curvirostris. As this parasite occurs in a different species of host, we may perhaps assume for the present that it is a form distinct from K. lacertæ, though I do not think this can be regarded as at all certain. A few weeks later, Nicolle [24] also described a similar Hæmogregarine, from a variety

of L. ocellata in Tunis, which he considered to be distinct from curvirostris and called biretorta. Lastly, França [7], not content with these two, makes three additional species, H. [K.] schaudinni, nicollei and minuta, to say nothing about a variety africana of his first one, all from L. ocellata. Thus in two species of Lacerta, namely muralis and ocellata, there are according to França no less than ten species of Hæmogregarine. Is not this carrying species-splitting to an absurd degree?

I have not studied the parasites of L. ocellata myself, but having regard to the above analysis of the so-called species of L. muralis, some of those from this other lizard must be viewed with great suspicion. For instance, biretorta is almost certainly the same parasite as curvirostris, and hence a synonym of the latter; this is clear to my mind, from França's figs. 15 and 17 (loc. cit.), and, indeed, Laveran and Pettit, in a note I have not been able to see,[1] have also thrown doubt upon the independent nature of biretorta. The same conclusion applies to França's species nicollei, which the author himself admits has considerable resemblance to curvirostris and biretorta; in short from França's fig. 18 it is obviously only a slightly different phase of K. curvirostris. The parasite termed by França schaudinni appears rather different in character both from lacertæ and curvirostris, although França's fig. 2 of this form is remarkably like my fig. 4 of K. lacertæ; it may perhaps be left an open question whether schaudinni is some other phase in the developmental cycle of K. curvirostris or a distinct species. It is rather odd, however, that França has included as a particular form of curvirostris a type (vide his fig. 16) which is undoubtedly only a form of his schaudinni! I conclude the subject by registering a strong protest against this habit of creating a new species on entirely insufficient grounds.

[1] 'Bull. Soc. Path. exot.,' ii, 1909, p. 377.

III. Comparison of the Nuclear Condition in Karyolysis lacertæ and Certain other Hæmogregarines with that of Coccidia; the Question of the Karyosome and the Intra-nuclear Division-centre.

I propose next to compare the nuclear condition, as I have described it above in Karyolysus lacertæ, with that which is found in certain Coccidia, at a particular period in the life-cycle, since, in my opinion, the agreement shown affords an important indication of the close affinity and phylogenetic relationship of these two types of parasite. This resemblance is especially marked in the case of the merozoites and very young schizonts of a Coccidian, which is, according to Shellack and Reichenow (**32**) really Barrouxia alpina, Léger; this phase, it must be mentioned, has for long been mistakenly included in the life-cycle of Adelea ovata, of which it was considered to represent the male type of schizogony. The structural details of this particular stage or form of the parasite were first described by Siedlecki (**33**), and further notes with regard to it have since been given by, among others, Jollos (**12**), both these authors having included it in the cycle of Adelea.[1] In order to facilitate the comparison with the Hæmogregarine, I have drawn (Pl. 9, figs. 41–43; Pl. 10, figs. 1–3) some individual merozoites from an original preparation of my own, these parasites being easily obtainable in centipedes. Although I have found exactly the same nuclear condition and behaviour in this early phase which has been observed by Jollos, I think it is worth while to describe it again, because doubts have been recently cast upon Jollos' account, both as regards these points and others.

At first the young schizont of Barrouxia, which may be

[1] The two forms are parasitic in the same host, Lithobius forficatus; this fact is, of course, chiefly responsible for their different phases having been confused together.

little more than a merozoite,[1] has a single large karyosome placed quite at one side of the general nuclear substance; the latter is finely granular in character, and does not stain deeply, the granules being fairly uniform in size and appearance. More frequently a rather later condition or phase is found, in which there are two karyosomes, generally at opposite sides of the principal nuclear mass; these two karyosomes are usually more or less unequal in size, and neither is so large as when there is only one. I have been much exercised in regard to the question of the true situation of these karyosomes. In nearly all the individuals a well-marked clear zone, which in some cases is relatively wide, surrounds both the general nuclear substance and the karyosomes (or karyosome). Is this clear zone to be considered merely as a shrinking-space, separating the whole of the nuclear organellæ from the general cytoplasm of the parasite, or is this area really within, and therefore a part of, the nucleus, the limit or border of which is on the outer side of the clear area and in contact with the edge or margin of the surrounding cytoplasm? In the former case, of course, the karyosomes would be actually extra-nuclear; in the latter they would be within the nucleus, but excentrically placed, near the periphery. After some hesitation I have come to the conclusion that the latter view is the correct one, and that the pale, clear area really constitutes the peripheral region of the nucleus. In the case of most individuals I have found it almost impossible to satisfy myself of the existence of a definite membrane, bordering this zone externally, as distinct from the edge or margin of the surrounding cytoplasm itself; and the same difficulty has presented itself apparently to other observers, if one may judge from certain of their figures (e.g. Siedlecki's fig. 17 and Jollos' figs. 22 and 28). Moreover, the limit of the centrally situated, uniformly granular,

[1] The earliest change in the condition of the karyosome, namely its division into two, may even take place before the fully formed merozoite has been liberated from the "barillet" of which it has constituted a segment.

nuclear material is at times so sharp and well-defined that it might almost be regarded as a membrane. However, now and again one is fortunate enough to be able to focus a definite line bordering the pale area in question on the outside, which most probably represents a true nuclear membrane. And there are one or two other reasons which support this view. Thus Siedlecki (loc. cit.) states that he observed this clear zone in these forms of the parasite even in the living condition, which shows that, in the strained preparations, it cannot represent merely a shrinkage-space. Further, although this zone appears so clear and pale by comparison with the parts of the parasite immediately surrounding, it is, nevertheless, occupied by something—probably in the nature of nuclear sap—which is extremely faintly stained; that this is actually the case is sometimes shown distinctly because of a peculiar condition or appearance which is often, but not always, presented by the karyosomes. These elements themselves, especially the larger ones, i.e. when there are only one or two, may be surrounded by a perfectly clear halo-like circle, which is quite colourless; this halo round the karyosome passes between it and the central nuclear substance, indenting the surface of the latter, so that it forms a concavity or cup as it were. The difference between this small, quite colourless zone and the almost clear, faintly staining area, extending around the periphery of the whole nucleus is sufficiently conspicuous. To sum up the matter, therefore, the karyosomes must be considered as really intra-nuclear, situated in a peripheral zone, which is very pale, and apparently consists only of nuclear sap, the rest of the nuclear material, containing a small amount of chromatin being aggregated to form a central mass. I have not been able to see any delicate threads or rays passing from this central mass to the limiting membrane of the nucleus, and traversing the faintly-stained, peripheral zone, nor does Jollos (loc. cit.) mention or figure anything of the kind; but Chagas (5) has described and figured "linin threads," having such a disposition in the case of somewhat older phases of a new species of

Coccidian ("Adelea" hartmanni),[1] in which the nuclear constitution and behaviour of the young schizont is very similar (cf. also below, Note IV, where the nucleus of Leucocytozoon is compared).

To return now to the behaviour of the karyosome. The two subequal or unequal karyosomes result undoubtedly from the division of the original large, single karyosome, which takes place in a promitotic manner; for in a couple of instances I have found the centrodesmose still persisting (cf. fig. 42). There is no possible doubt about this division of the karyosome; the process here appears to be just the same as in Karyolysus lacertæ, and my having found it in both parasites substantiates and corroborates Jollos' account of this behaviour of the karyosome in the young schizonts of this Coccidian. While the early condition and behaviour of the karyosome during this period is thus completely paralleled by the above-described early phase of K. lacertæ, the subsequent course of events differs slightly in the two parasites. In the Coccidian, at a rather late stage, three or four karyosomes are present (fig. 43, also fig. 3, Pl. 10), most of which are small and have obviously arisen by the further division of one or both of the two above-mentioned daughter-karyosomes (cf. also Jollos' figure).[2] That is to say, here the karyosome continues to be separate and distinct from the general nuclear substance (as is known from the ascertained further development), whereas in K. lacertæ the karyosomatic chromatin which is retained by the nucleus becomes distributed amongst the general chromatic substance and no longer distinguishable.

It is necessary to emphasise this fact of the promitotic division of the karyosome because, in recent papers, Reichenow

[1] This parasite is regarded by Léger (16) as the type of a new genus, Chagasia.

[2] It may be recalled that Siedlecki himself, in his original description of this form, also states that the karyosome divides: thus, "il [le karyosome] donne, par bourgeonnement, naissance à des karyosomes secondaires," and, again, "surtout un karyosome, parfois divisé en deux ou trois fragments."

(27) and Schellack and Reichenow (32) have maintained that no division of the karyosome occurs in the above phase of Barrouxia ("Adelea"), and consider that the secondary karyosomes (i.e. the daughter-karyosomes) arise de novo, by independent formation from the general nuclear substance; in regard to this detail the authors are certainly mistaken. Moreover, quite recently Chagas (loc. cit.) describes and figures, in his account of Chagasia (Adelea) hartmanni, perfectly similar promitotic divisions of the karyosome in different phases of the life-history. I have a strong idea that Reichenow and Schellack, in arriving at the above conclusion, have been influenced—if unconsciously—by the view which one of them (Reichenow) seems to have formed upon the question of the karyosome, its nature and significance, as a result of his work on Hæmogregarina stepanovi. No one is more sensible than am I of the great value of Reichenow's research, which has thrown full light upon the complicated subject of the Hæmogregarine life-cycle; but in regard to this somewhat important cytological question I find myself obliged to differ from him.

Hartmann and Chagas (10) have suggested that the reason for this may be that as the particular parasite (Hæmogregarina stepanovi) upon which Reichenow worked is a very small one, the observation of minute cytological details and changes would be rendered more difficult and hence they may have escaped detection. I do not altogether share this opinion; for one thing, I do not think H. stepanovi is much, if any, smaller than the small forms of K. lacertæ, where the karyosome and its division can be made out without difficulty. I am inclined to consider that, on the whole, the nuclear constitution and behaviour in H. stepanovi is as Reichenow has described it; and therefore, as a logical sequel, that this species of Hæmogregarine differs in one or two cytological respects, such as the absence of a typical karyosome, from certain other Hæmogregarines and certain Coccidia. This is the more probable, in my opinion, because of a fact which is evident on scrutinising Reichenow's figures, namely, that the

chromatin of the general nuclear substance is very much more prominent, i. e. in the form of numerous fairly large, deeply staining grains, than is often the case in the corresponding phases of other parasites where a karyosome is present; and just the same condition is seen in the closely allied species, H. nicoriæ, according to Miss Robertson's description (loc. cit.). If the nuclear appearance of these parasites is compared with that, for instance, of the young phases of either K. lacertæ, H. gracilis (Wenyon [36]), H. lutzi (Hartmann and Chagas [10]), or of Barrouxia alpina ("Adelea ovata") or Chagasia hartmanni, a striking difference is at once apparent; in the latter, most, sometimes nearly all, of the chromatin is contained, for the time being, in a distinct karyosome (or more than one). It is especially in regard to this absence of a definite karyosome that the two species of Hæmogregarine from tortoises are interesting. Thus, Miss Robertson expressly states that "at no stage does H. nicoriæ show in its nucleus the karyosome so characteristic of Coccidia." Now, in my opinion, H. stepanovi shows an important intermediate condition between the type of nucleus possessing a karyosome, as in the above examples, and a type like that of H. nicoriæ, where this organella is quite wanting. According to Reichenow, H. stepanovi has at certain periods of its life-cycle (which, in general, correspond to the phases when a karyosome is present in other forms) a definite rounded body, situated near the periphery of the nucleus, which is always very pale and faintly stained and appears quite different from the prominent chromatic grains.

Reichenow uses the term "nucleolus" for this body, and this is most probably the correct name for this particular structure, and indicates its true nature; but my reason for thinking so is not exactly the same as that given by Reichenow. It seems clear from the author's description and figures that the body in question contains little or no chromatin; it corresponds apparently to the true nucleolus of an ordinary tissue-cell, i.e. a body consisting simply of

plastin or allied material. Reichenow, however, regards this element as a nucleolus principally on the ground of its behaviour during nuclear division, that is to say, its disappearance and re-formation at different periods. Unfortunately, Reichenow's observations on this body in H. stepanovi, which have led him to the conclusion that it has the physiological significance of an ordinary nucleolus, have prejudiced his view upon the true karyosome, which is something quite different. He has, in my opinion, failed to grasp what is the really essential feature of a true karyosome, namely, that it is a chromatin-nucleolus, an organella which holds or contains a large proportion of the entire chromatic substance of the nucleus. His only reference to this fundamentally important character is seen in the following sentence:—"Was ihn [d. h. den Binnenkörper (Karyosom)] von dem echten Nucleolus unterscheidet ist, abgesehen von seinem Chromatingehalt auf den wir keinen grossen Wert legen dürfen, allein der Umstand, dass er bei der Kerntheilung erhalten bleibt" (the spacing is mine). Because he thus ascribes no importance to this, the principal feature of the karyosome, he is able to persuade himself that the typical "Binnenkörper" or karyosome in other cases is the equivalent, practically speaking, of the body he has described in H. stepanovi.

Further, Reichenow brushes aside as quite untenable the usually accepted view that the karyosome behaves as an intra-nuclear division-centre, which is founded on the reliable observations of many previous workers. The admitted existence of the "Hantel-Figur" he endeavours to explain by supposing that it is produced by the karyosome being drawn out into two parts by the separating halves of the dividing nucleus. He appears to have adopted this attitude on two grounds: in the first place, because he has found that the nucleolus of H. stepanovi does not so divide, and secondly, because he evidently doubts the existence at all of an intra-karyosomatic centrosome and the occurrence of promitotic division, so far as the Coccidia and Hæmosporidia are con-

cerned. In regard to the first point, the very fact that the organella seen in H. stepanovi is a nucleolus and not a karyosome explains why it does not divide, as I hope to show below (cf. pp. 213 and 214).

With regard to Reichenow's doubts about the occurrence of promitotic division and the presence of an intra-karyosomatic centrosome, I must say I think they are quite unfounded. In the first place, both my own observations on the same Coccidian and those of Chagas on an allied form support Jollos' account (loc. cit.) in so far as regards this detail. Further, I have found a precisely similar division of the karyosome by means of a centrodesmose in an early phase of the Hæmogregarine, Karyolysus lacertæ.[1] And, as I have previously remarked, the presence of a centriole within the karyosome may be legitimately and reasonably assumed where the occurrence of a centrodesmose is noted. From a study of Trypanosomes, I know how difficult it often is to actually distinguish the centrosome, even in the large karyosome of a relatively large individual, although the occurrence of a centrodesmose in the division of the karyosome (e. g. of the trophonucleus) has long been well known. Nevertheless, Minchin and I, in our notes on T. raiæ (**20**), clearly demonstrated the actual presence of a centrosome in the resting karyosome. Moreover, as regards the Hæmogregarines, since Reichenow's paper appeared, some interesting observations on the leucocytic parasite of the dog, Hepatozoon (Hæmogregarina) canis, have been published by Wenyon (**37**). Here, too, a distinct promitotic division of the karyosome is figured; and in the case of this parasite, the karyosome is relatively very small in some phases, when it probably represents little more than the centrosome itself. Even in the nucleus of H. stepanovi, it is not impossible that a centriole is really also present, and it is just in regard to this detail that I think the suggestion of Hartmann and Chagas (**10**) may apply, namely, that this minute granule may have escaped recognition owing to the difficulty of distinguishing it amid the

[1] Cf. also footnote to p. 205.

more prominent chromatic grains. In this connection it must be noted that Miss Robertson (**28**) mentions and frequently figures a small but definite granule in the nucleus of H. nicoriæ, which is in no way distinguishable from the peripheral chromatin grains in size or staining reaction, but which nevertheless appears to be different from the other nuclear elements in so far that, in the primitive type of nuclear division, it seems to form a centrodesmose. This minute body may well be the centrosome; just as the central granule which I have sometimes noted in the nucleus of K. lacertæ, when there is no longer a distinct karyosome, is also probably one (cf. fig. 40).

It is a pity that Reichenow, in his able memoir, should have thought himself at liberty to disregard or treat as negligible the evidence afforded by the research of other earlier workers, such as the classic instances of Coccidium schubergi and Cyclospora caryolytica, made known by Schaudinn (**30** and **31**), which pointed clearly to the existence of this characteristic promitotic division of the karyosome in the respective parasites, and which has since been abundantly corroborated in other cases; to say nothing of his having entirely failed to take into consideration that in several of the lower Flagellates the occurrence of a centrosome and of promitotic division of the karyosome is now well established. As it is generally agreed to-day that the Ectospora (Telosporidia) are descended from Flagellate ancestors, it might be expected, on à priori grounds alone, that among Coccidia and Hæmosporidia some would be found to exhibit a similar mechanism in their nuclear division.

I certainly do not think it is advisable to adopt such a comprehensive generalisation as that postulated by Hartmann and Chagas and the followers of their school, namely, that a central organella (centrosome) is present, as a general rule, in the karyosome of all Protozoa; but I will at once admit that I consider this idea considerably nearer the truth than the view maintained by Richenow, that a centrosome is not present in the karyosome in any of the cases mentioned

above, and that no promitotic division of this body occurs.[1]

Nature and Significance of the Karyosome.

I have laid stress upon this fact of the presence of an intra-karyosomatic centrosome because of the important bearing it has upon the question of the real nature and significance of the karyosome, and because it helps to explain satisfactorily the different behaviour of this body in different phases of the life-history. In the first place, it is necessary to clear the ground of what I consider is a serious misconception of the karyosome, which is largely fostered by the school of Hartmann, Nägler and others, and which appears to be based upon the fact that this organella frequently leads the way in nuclear division, and contains within itself a division-centre. Now, the primary and principal meaning of the term karyosome is chromatin-nucleolus, i. e. a body consisting of a plastin basis impregnated with chromatin; it might be considered unnecessary at the present day to have to emphasise this essential character, but that this is not so is shown by Reichenow's reference to it as one "auf den wir keinen grossen Wert legen dürfen!" This is the sense in which the word was first used, and on account of which it has been adopted by most authors (cf. Labbé (13), Minchin (19), Siedlecki (33 and 34), Wilson (38) and others). Schaudinn, in his celebrated memoir on the Coccidia of Lithobius also says: "Jeden Falls unterscheidet sich das Karyosom der Coccidien von den echten Nucleolen der Metazoenzellen scharf durch seinen Chromatingehalt." But in many recent papers by members of the school of thought referred to above, a strong tendency is noticeable to assume that the possession of a centrosome and of the function of acting as a division-centre is to be definitely associated with the idea of a karyosome as a whole and to be implied in the meaning of the

[1] See also the account given in Note IV of the nuclear structure of Leucocytozoon and Halteridium, in both of which division-centre and centrodesmose are clearly shown.

term, as a definite attribute of this body; thus, Hartmann and Chagas (11) say: "Man kann daraufhin jetzt den Begriff des Karyosoms direkt von dem Vorhandensein eines Centrioles [Centrosoms] abhängig machen." This notion has been elaborated to such an extent that the whole karyosome, that is to say, chromatin-nucleolus + centrosome, has come to be regarded as a distinct entity, a locomotor or kinetic centre; its chromatin is the "kinetic component," while the surrounding chromatin, scattered through the nucleoplasm or nuclear sap, is the "generative component" (the second nuclear type of Hartmann and Chagas).

Now, in my opinion, this idea of the karyosome is very forced, besides being really quite unsupported by any evidence. For one thing, I do not consider that the whole karyosome (i.e. chromatin-nucleolus + centrosome) can be regarded as representing a definite unit or "locomotor-centre"; it may happen, in fact, that the intrinsic division-centre is outside and distinct from the karyosome (as in Spongomonas, for example, figured by Hartmann and Chagas, and cf. also the "nucleo-centrosome" of Adelea zonula, according to Moroff (21). Again, the condition shown by the true Binucleata, the Trypanosomes and their allies is quite against this interpretation. Here there are two separate nuclei—a locomotor nucleus (kinetonucleus) and a vegetative one (trophonucleus); to this, of course, Hartmann and Chagas assent, saying (loc. cit.) that "zwei verschieden differenzierte Kerne in der Zelle vorhanden sind, einer [trophonucleus] vorwiegend mit der trophisch-generativen Komponente, der andere [kinetonucleus] vorwiegend mit der lokomotorischen Komponente." But nothing is more certain than that the trophonucleus of a Trypanosome possesses a large, conspicuous karyosome, containing most of the chromatin of the nucleus, and also a distinct centrosome (centriole)! If, therefore, the karyosome in this case is a trophic component (which is, indeed, the most reasonable view to take), whatever ground is there for supposing that, in the passive, intra-cellular Coccidian, the equally large and

conspicuous karyosome represents a kinetic (locomotor) component? Moreover, another idea prevalent in the writings of the adherents of this school, which is strongly to be deprecated, is that of contrasting, as two opposed constituents, kinetic and generative components of the nucleus. These two things are not strictly comparable or opposable at all. On the one hand, the essential kinetic components are the achromatic elements —centrosome, centrodesmose, and so on; and in all probability these take part in effecting the division of generative chromatin as well as of vegetative (trophic) chromatin. And, on the other hand, where a separate kinetonucleus is present, which may be regarded as standing in a special relation to the locomotor activities of the Trypanosome, there is no reason whatever for supposing that the chromatin of this nucleus is less generative in character than that of the trophonucleus.[1] In short, I cannot share the above view of the locomotor or kinetic nature of the karyosome as a whole at all; it is the contained centrosome, not the chromatin-nucleolus, that brings about the division. The so-called "Hantel-Figur" is really the result of the gradual (passive) separation of the two halves of the karyosome as the centrodesmose extends.[2]

It seems to me very much better to return to the earlier manner of regarding the karyosome, which has been well set forth and discussed by Siedlecki (**34** and **35**), namely, that it is an organella, whose principal function is to store up reserve chromatin—and particularly trophic as distinct from generative chromatin—for use as and when required by the nucleus, or, as the case may be, for elimination if not required. This theory undoubtedly fits in best with the known variations in

[1] This point was emphasised by me so long ago as 1906 in my analysis (40) of Schaudinn's celebrated work on the parasites of the little owl.

[2] The same interpretation is in all probability to be applied to the "nucleolo-centrosome" (Kenten) of Euglena, especially as Hartmann and Chagas (loc. cit.) have shown that promitotic division of the karyosome, by means of a centrodesmose, occurs in another Englenoid, Peranema trichophorum.

behaviour of the karyosome at different periods of the life-cycle. For instance, as regards the Coccidia, speaking generally it may be said that during the schizogonic, vegetative phases, the karyosomatic chromatin becomes subdivided up, in a promitotic manner, amongst the daughter-individuals; on the other hand, as a rule, on the approach of the sporogonic part of the cycle—frequently during gametogony or else early in the history of the zygote—the karyosome is mostly eliminated, a "nuclear purification" of the unrequired, trophic chromatic material taking place.[1] Moreover, in connection with this view, a very simple explanation can be offered of the presence of an intra-karyosomatic centrosome, one which appears to me to render quite unnecessary the involved conception of the karyosome discussed above. It must be remembered that the promitotic type of division, which is the type found where the centrosome is contained within the karyosome, is of a primitive character, as its name implies. It is most likely that the reason why the centrosome, i.e. the intra-nuclear division-centre, is inside the karyosome in such cases is simply because the latter body does contain, for the time being, the larger proportion, or it may be nearly all of the chromatin of the nucleus, the division of which it is the function of the centrosome to bring about and regulate; in other words, because, having regard to the primitive character of the mechanism, the function of the division-centre is the better performed the more intimately it is associated with the chief chromatin-containing constituent of the nucleus.

Further, on this view a separation of centrosome and karyosome, as the nuclear development reaches a slightly more advanced stage, would be readily intelligible. Such an occurrence of the division-centre distinct from, or independent of, the karyosome (but at first, of course, remaining intra-nuclear) may have been brought about in more than one way. Thus it may be the result of a more elaborate develop-

[1] It may be noted that Léger and Duboscq (17), in their admirable account of the sexual processes among Gregarines, also adopt this interpretation of the elimination of karyosomatic material.

ment of the mechanism of division; an example of this is seen in the case of Spongomonas, to which reference has previously been made, where the centrosome passes out of the karyosome at the period of division and a definite mitotic figure is formed. Or, on the other hand, it may be due to the development of another type of nuclear structure, where, either during certain phases in the life-history or throughout the whole cycle, there is no longer a karyosome present in the nucleus as such, but the chromatin is more or less uniformly distributed on a reticulum throughout the general nuclear substance, in the middle of which the centrosome may persist.[1] And it is in this direction that the nuclear constitution has apparently developed in the Hæmogregarines. Lastly, a further stage in nuclear evolution would be reached by a combination of the two lines of development indicated, i. e. by the elaboration of the nuclear structure itself, associated with a more perfect development of the division-mechanism; and thus a condition might be arrived at such as is seen in the daughter-nuclei formed during the period of nuclear multiplication, which precedes gamete-formation, in many Gregarines (cf. the figures of Brasil [4], Léger and Duboscq [loc. cit.], Woodcock [39]), where we find perhaps the highest grade of nuclear constitution and mode of division attained among the Sporozoa.

[1] It is important to note that even where a division-centre is certainly present during particular phases of a life-cycle, this may nevertheless be wanting, or at any rate not recognisable, during other periods of the same life-cycle. Thus, in many Coccidia (e.g. Coccidium schubergi, Cyclospora karyolytica, according to Schaudinn), the division of the definitive nucleus of the zygote to form the sporoblast-nuclei is direct; but, on the other hand, in Adelea (cf. A. ovata, mesnili [Perez, 25] and hartmanni) the sporogonic divisions appear to be promitotic, i. e. more or less comparable to the schizogonic ones, allowance being made for the absence of a karyosome). Again, in the nuclear divisions of the sporont of the Gregarine, Diplodina irregularis, I have shown (39) that the first ones are direct (amitotic), the later ones mitotic.

We are now in a position to summarise, comparatively, the various types of nuclear condition which have been described in different Hæmogregarines. In Karyolysus lacertæ a definite karyosome is present in the youngest schizonts. This undergoes promitotic division which is usually unequal. The smaller half divides again, and the resulting portions ultimately become incorporated with the general nuclear material; the larger half of the karyosome, on the other hand, is eliminated from the nucleus and passes to the surface of the body-protoplasm, becoming altered and probably partially used up by the cytoplasm in its passage.[1] As already mentioned, I am of the opinion that the division-centre persists in the modified nucleus and can be seen at times as a definite central granule. I am unable to say whether a karyosome is developed again in a later phase of the life-cycle. In Hepatozoon (Hæmogregarina) canis, according to Wenyon (37), the karyosome persists throughout the schizogony, its division occurring in the usual promitotic manner; in this case, the body regarded by Wenyon as a karyosome is very small comparatively, and, I should say, represents little more than the intra-nuclear division-centre itself. Wenyon does not mention whether he observed any elimination of chromatic material before or during schizogony. On the other hand, in Hæmogregarina nicoriæ a karyosome cannot be distinguished at all, the nucleus appearing in all phases to have its chromatin more or less regularly distributed upon a reticular framework; a definite intra-nuclear centrosome is regarded, however, as being present. H. stepanovi shows, as I consider, a very interesting stage in the disappearance of the karyosome as a distinct organella. In certain phases a nucleolus is present,

[1] It is instructive to note that a similar elimination of karyosomatic material before the young schizont proceeds to nuclear multiplication is described by Averintzeff (1) in the case of Barrouxia sp., parasitic in Cerebratulus. The process may apparently take place according to one of two slightly different modes, the second of which furnishes a close parallel to the nuclear behaviour of the corresponding phase in Karyolysus.

occupying the same excentric or peripheral situation in the nucleus which is occupied in other forms (e. g. Karyolysus, "Adelea") by the karyosome. I suggest that this element represents the plastin basis of an ancestral karyosome, the chromatin which it originally stored having become now (permanently) distributed through the general nuclear material in the form of numerous large conspicuous grains.[1] In this connection an observation made by Reichenow is significant. He found that in the young growing schizont, chromatic substance is regularly eliminated from the nucleus and cast out of the cell-body of the parasite, i. e. a precisely similar occurrence to that seen in Karyolysus and Barrouxia sp. Reichenow is uncertain whether it is the nucleolus ("Binnenkörper") which is thus got rid of; but, as he himself points out, the fact that the nucleolus is always very faintly stained, while the expelled element stains on the contrary deeply and is manifestly chromatic in origin, is against this view. Moreover, I may point out that in slightly older schizonts again, the nucleolus is still present in the nucleus (cf. Reichenow's figs. 73–75). Hence it is more probable that this eliminated chromatic substance is derived from the general nuclear chromatin. As this process here doubtless has the same object as the corresponding one in other parasites, the inference is that the chromatin which in other cases is stored up in the karyosome is in H. stepanovi incorporated with the rest of the chromatic material of the nucleus, the plastin basis of the karyosome alone remaining. On this explanation, and having regard to the views I have expressed above, it is readily understandable why the nucleolus does not divide, with the formation of a "Hantel-Figur," a fact which appears to have puzzled Reichenow. There is no need for a division-centre to be present in the nucleolus because it no longer possesses the chromatin of a

[1] So prominent are these grains and apparently in certain phases usually of a fairly constant number (i. e. within limits) that Reichenow is inclined to regard them as definite units comparable to chromosomes.

karyosome (chromatin-nucleolus). If, as seems to me quite possible, a centrosome does occur in H. stepanovi, this is most likely to be in the centre of the chromatic network of the nucleus.

Before concluding this section, I should like to add a few remarks about the nuclear condition seen in the piscine Hæmogregarine, H. triglæ, to make a comparison with which was my original intention in commencing to study the nucleus of K. lacertæ. Minchin and Woodcock (loc. cit.) found that in both the small forms and the two large types of the parasite one or two large grains are frequently, though not invariably present, situated either close to the nucleus, or some varying distance from it; these bodies are very deeply stained and prominent in films stained by iron-hæmatoxylin. The nucleus itself appears comparatively pale and consists of small grains of chromatin, often somewhat faintly stained, on an irregular network. In Giemsa-stained smears it is difficult to distinguish this grain (or grains) when close to the nucleus. In our paper describing H. triglæ we regarded these elements as not chromatic, but rather of the nature of centrosomes. The extra-nuclear position of the body, together with the fact of its being often paired, seemed to us very much against its representing a karyosomatic element. Moreover, the appearance of these grains after being stained with Twort's stain did not, in our opinion, furnish sufficient evidence in favour of their being chromatic. It is true that in freshly made preparations they were often stained red, i.e. with the neutral red, the chromatin staining constituent of Twort; but they had no strong affinity for the red, because in preparations which had been made some time the red tint had quite vanished from them, although the nucleus itself retained the red colour. I think we were misled by this behaviour after Twort. While it may be said that only chromatic elements are stained red by this stain, I think now that it is nevertheless quite likely that chromatin in some states or conditions may possess only very slight affinity for the neutral red.

Discussing at the time the question of the Hæmogregarine nucleus, we considered this to be of a distinct type, entirely lacking a karyosome. Börner (**3**), in his account of Reptilian Hæmogregarines (the best, as regards cytological details, which had been published up till then), had expressly stated that he never in any case found a karyosome present. Moreover, mentioning the matter in conversation with Miss Robertson, she also agreed that the Hæmogregarine upon which she was at the time working (H. nicoriæ) also had no karyosome associated with its nucleus. The only mention in the literature up to then of the occurrence of a karyosome in the nucleus of a Hæmogregarine was by Wenyon (**36**), in the case of certain phases of H. gracilis, from the liver of Mabuia. It appeared to us at that time highly probable that Wenyon had mistaken phases of some Coccidian parasite of the liver for phases of the Hæmogregarine, particularly as other, rather similar stages figured by Wenyon, which were undoubtedly referable to the life-cycle of H. gracilis, showed no karyosome in the nucleus. In the light of the observations discussed in the present paper, I willingly admit that our opinion was very probably mistaken, and that Wenyon may have been quite right in attributing all the phases he figured to the life-cycle of H. gracilis.

In short, it is now perfectly clear that the Hæmogregarine nucleus cannot be considered as being of a distinct type, but that, on the contrary, it shows close agreement with, or is easily derivable from, the Coccidian nucleus. Either a definite karyosome is present, at all events during some part of the earlier (schizogonic) phase of the life-history, when it behaves in a manner quite parallel to what is found in certain Coccidia, or else its complete absence is readily accounted for by a consideration of its behaviour as the development proceeds in those parasites in which it does occur.

Therefore, in the case of H. triglæ, it is most probable that the conspicuous grains also represent karyosomatic elements, and that they do contain chromatin in some form or other. In our preparations we did not observe any division-

figures, but it is not unlikely that where two grains are present, they have originated by the division of one, if comparison is made with the somewhat similar condition seen in Kalyolysus and "Adelea." Whether, again, a portion of the chromatic material is used to replenish the chromatin of the reticulum, or whether it is all unrequired and eliminated, I am unable to say. No definite centrosomic granule was noticed within the nucleus itself.

Note to Part III.

Since this part was written my attention has been called to an important paper by Debaisieux (6A), on the Coccidia of Lithobius. I am only able here to indicate briefly the conclusions arrived at by this author, in so far as they bear upon the chief points which have been considered in the above section. Debaisieux also finds, as do Schellack and Reichenow, that phases of more than one parasite have been confused in previous descriptions of Adelea ovata. No reference whatever is made, however, to Schellack and Reichenow's note—an omission which is to be regretted. Debaisieux agrees that there is no double (or sexual) schizogony in the true Adelea ovata; but whereas Schellack and Reichenow refer those phases which do not belong to Adelea to Barrouxia alpina, Debaisieux refers them (at any rate, those observed by Jollos) to Coccidium lacazei. I am very pleased to find that Debaisieux also entirely upholds the occurrence of a true division-centre (centrosome) and of promitotic division of the karyosome, as described by Jollos (loc. cit.); though it may be mentioned that, as regards the precise modes of nuclear behaviour and division in the later stages of schizogony, he differs in certain points from that author. Further, Debaisieux takes a view upon the nature and significance of the karyosome quite similar to that which I have mentioned above; and this author also dissents from the ideas about the karyosome propounded by Hartmann and his school.

IV. The Nuclear Structure of Leucocytozoon and Halteridium; the Significance of the So-called Binucleate Condition in these Forms, and its bearing upon the Affinities of the Hæmosporidia.

The observation of the occurrence of a distinct karyosome in certain Hæmogregarines led me to study again, from this point of view, the much-discussed nuclear condition found in the gametocytes of Leucocytozoon and Halteridium. As is now well known, female individuals of both these parasites, when stained by some modification of the Romanowsky method, show besides the ordinary nucleus, which is stained red, another very definite nuclear body, which stains much more deeply than the other, and at times appears almost black; this additional chromatic element may be either close to (in contact with) or quite separate from the nucleus. In the case of Halteridium this body has, in female individuals, the form of a conspicuous grain, but in the distinctive individuals which have been regarded as neutral or "indifferent" (which, it may be incidentally remarked, seems to occur only rarely), it is even more prominent and may be almost as large as the nucleus. In the case of male individuals, however, I have not succeeded in making out anything comparable to this structure. As I have previously described and figured the appearance shown by Halteridium fringillæ, when stained by Giemsa, I need not refer further to it; I have found exactly the same appearance in Halteridium noctuæ of the little owl.

In the case of Leucocytozoon ziemanni, the celebrated Leucocytozoon of the little owl, the additional chromatic body is very large and prominent in the female gametocytes (Pl. 10, figs. 4–6), and by no stretch of imagination can it be regarded merely as a grain! Anything more like the trophonucleus and the kinetonucleus of one of the large "blue" Trypanosomes present in the same bird might be expected to appear, in a resting, intra-cellular condition, it is impossible

to suggest; and I well remember that when I first saw such individuals in preparations, really well-fixed and stained,[1] I felt no more doubt that Schaudinn's view would prove to be correct than I felt about being at Rovigno. In my opinion, this remarkable resemblance was the foundation upon which Schaudinn built up his whole theory of the ontogenetic connection between the Trypanosomes and the intra-cellular parasites (Leucocytozoon and Halteridium) of the little owl. To return to L. ziemanni, in the male gametocytes the great majority here also show no chromatin body in Giemsa-stained smears besides the large, oval, diffuse nucleus, the scattered granules of which stain faintly a pale red (fig. 7). Occasionally, however, two or three small bodies or grains, which may differ slightly in size and which stain red somewhat more deeply than the nucleus, can be made out situated close together near the margin of the nucleus, forming as it were a clump almost in contact with it (fig. 9). These small structures are really only conspicuous in individuals which are if anything over-stained. Nevertheless the elements thus occasionally indicated in the nucleus of the male forms, stained by Giemsa, are found to be practically as constant in occurrence, in films stained by iron-hæmatoxylin, as is the single large body present in the female forms.

[1] This remark is not made with any idea of self-praise; it is by no means an easy matter to obtain Leucocytozoon well fixed and stained, even according to the Romanowsky method, so as to show the nuclear structure properly, and also the different parts of the host-cell, in their true form and relation to the parasite. It is only necessary to glance at many of the figures of different species of Leucocytozoon hitherto published to realise this. Either the parasites are hopelessly distorted and flattened out (cf. Dutton, Todd and Tobey's figs. [2]), or the only sign of a nucleus is a space-like area in the middle of the cytoplasm (as in some of Mathis and Léger's recent figures [3]); some of Wenyon's figures, too, of L. neavei (8) are far from giving an accurate representation of the form and nuclear details. My figures in the present paper, as also those of L. fringillinarum in a previous memoir (9), show approximately the true nuclear appearance, as will be seen when the condition found in wet-fixed preparations stained by iron-hæmatoxylin is discussed.

The study of these gametocytes of Leucocytozoon in films stained with iron-hæmatoxylin is most instructive. Berliner, in his account of Flagellates (1), has also given figures of the intra-cellular parasites, Leucocytozoon and Halteridium, stained in this manner, with the idea of showing that they agree with the Binucleata in the possession of two nuclei (i.e. the occurrence of nuclear dimorphism); he does not, however, give any description of the details of nuclear structure. As regards the female forms, the figures given by Berliner show, on the whole, the same appearance as that which I have found.

Taking a general view, as it were, first, of the nuclear structure of the female gametocytes (figs. 11–17), this is seen to be, in many respects, of a similar type to that of the young schizonts of "Adelea." For the most part the nucleus consists of a fairly large, central mass, which appears finely granular and stains to a moderate degree; surrounding this the same clear, almost colourless zone can usually be made out, which is present in "Adelea" (cf. figs. 1–3). Berliner figures well-marked rays traversing this narrow zone; now and again I am inclined to think I have caught a hint of the presence of one or two of these rays, but in my preparations they are so faint and elusive that it is difficult to be certain. Standing out conspicuously by reason of the intensity with which it stains is the large chromatic body, which is so prominent in Giemsa-stained smears; this is always spherical and generally surrounded by a distinct halo, as is the karyosome in "Adelea." It is usually in close association with the nucleus proper, though it may be distinctly separate from the latter, as in fig. 11, but I have never seen it so far removed as I have found it in Giemsa-stained preparations (cf. figs. 6 and 8). In two or three cases I have observed two such bodies, of unequal size, and neither so large as when there is only one, lying at opposite sides of the central mass (figs. 12, 17); the resemblance of the nuclear condition in such cases to that seen in figs. 1 and 2 of "Adelea" and figs. 24, 26, Pl. 9, of

Karyolysus, in a preceding note, is striking. This occurrence is apparently infrequent,[1] but the observation of it has considerably helped to influence me in my decision to relinquish, as no longer tenable, the view I have formerly held respecting the origin and significance of this much discussed element. Regarding this body (or bodies) in the light of the nuclear constitution existing in certain phases of "Adelea," and especially bearing in mind the fact that I have myself made known above a similar karyosomatic condition in a blood-parasite, Karyolysus, the conclusion seems to be forced upon one that here also we have to do with a true karyosomatic element, and not, after all, with a body comparable to the kinetonucleus of a binucleate Flagellate.

In regard to the finer cytological points, the nucleus of these female gametocytes differs slightly from that of the early schizonts of "Adelea" and Karyolysus, as might indeed be expected when the different nature and subsequent development of the two types of individual is borne in mind. In those cases where there are two unequal-sized karyosomatic bodies (as I intend to designate these intensely staining elements in future), I cannot say whether they arise by the division of a single original one, in a primitotic manner, though I think this quite likely. I have not observed a spindle connecting them, but that may be because I have only found very few individuals in which there are two of these bodies. On the other hand, there is certainly a division-centre in connection with the central part of the nucleus, for not infrequently a distinct spindle (centrodesmose) is seen stretching between two granules, one of which stands out particularly from the more faintly stained chromatic material (figs. 11, 14). One of Berliner's figures also show this centrodesmose. The two granules connected by this spindle appear to be situated at the periphery of the central mass, and one is usually larger and more prominent than the other. In one instance I have observed a spindle running from the larger

[1] It is somewhat remarkable that, in Giemsa-stained smears, I have never noticed two of these structures associated with the nucleus.

granule to the karyosome (fig. 16). The larger granule may be present without there being any spindle or smaller granule (fig. 13). Very frequently close to this large granule is another one of about the same size and appearance; but this latter appears to lie always outside the central mass of the nucleus, at the outer edge of the clear, surrounding zone (figs. 14–16).

Turning now to the male gametocytes, there is always a large, oval nuclear area. As in Giemsa-stained smears, this is more usually very faintly stained (figs. 18–22)—remarkably so for a nucleus after iron-hæmatoxylin. It consists apparently of a loose reticulum with fine granules scattered throughout it. Here, also, this oval area is surrounded by a more or less distinct clear zone, but I have never, in this case, been able to make out any traces of rays crossing it, though Berliner (loc. cit.) just indicates a few in one of his figures purporting to be of male gametocytes. Berliner's figures of male individuals, however, are much less satisfactory than those which he gives of female ones; and, in fact, I am very much inclined to doubt their representing male forms at all, for reasons which I will mention shortly. In the majority of cases the outer limit of the nucleus, external to the narrow, clear zone, is more or less strongly impregnated with chromatin, in the form of distinct granules, which stain deeply, and in optical section constitute a prominent chromatic ring, sharply delimiting the periphery of the nucleus (figs. 20–23). It is noteworthy that this well-marked peripheral zone of chromatic granules in the male nucleus is apparently never to be observed in Giemsa-stained smears; it is not obvious in any of my preparations (for instance the individual of fig. 7 is on a smear made at the same time as the cover-slip preparation on which is the parasite of fig. 21), nor is it shown in any figures hitherto published. However, this zone is not always apparent, even in iron-hæmatoxylin preparations; thus the individuals of figs. 18, 19 do not show it. Although, of course, the intensity of staining and the degree of extraction

have a marked effect upon the prominence of this chromatic zone and the apparent size of the granules composing it, as equally upon the appearance of the host-cell nucleus (cf. figs. 22, 23), nevertheless I do not think the seeming absence of the zone in the instances mentioned is due, to any great extent, to the technique, i.e. to a less intense staining or to an excessive amount of extraction; for one thing, both the host-cell nucleus may be more intensely stained, and the host-cell itself, i.e. its spindle-like prolongations, more readily discernible, in cases where the nucleus shows no chromatic zone than in cases where it does (cf. figs. 18, 19, and 20, 22). Again, while all the preparations made from one infected owl may show the chromatic ring prominently, in those made from another bird this feature will be either not nearly so strongly marked, or else not discernible at all; this fact also points to a difference in this condition, in different cases or at different periods. I may emphasize the fact that I have never observed it in female gametocytes.

Almost constantly associated with the male nucleus is a group of small, spherical, deeply staining elements. Very generally these are three in number; a larger, more external one and two smaller ones, of approximately equal size. The larger body is situated at the edge of the nucleus, or just outside the border or periphery (figs. 18, 19), and is often surrounded by a distinct halo. Both in position and appearance this element agrees closely with the large, conspicuous body associated with the nucleus in the female gametocytes, the only apparent difference being that it is never so large; and I do not hesitate to suggest that it represents the same organella in the male forms, namely a karyosome. Why this chromatic element should stain so much more easily and intensely with Giemsa in the case of the female individuals than it does in the male forms is another instance of the peculiar and misleading vagaries of this stain. The two smaller elements I have mentioned, which apparently represent a pair, are situated at about the limit of the central

diffuse area of the nucleus, i.e. just internal to the narrow, clear zone (figs. 18, 19); (of course the disposition of these various organellæ can only be correctly ascertained when they happen to lie in the plane of optical section). The two granules are sometimes connected by a short but distinct spindle (fig. 22); and in one case (fig. 21) I have observed a spindle joining one of these granules to the larger body (karyosome).

It remains now to compare these granules occurring in the male nucleus with those described above in the female nucleus. It is highly probable that the pair of granules in the male form corresponds to the two approximately equal-sized granules seen in the female gametocytes of figs. 14–16, near the periphery of the nucleus. There is a marked agreement, moreover, between the nuclear condition shown in figs. 21 and 16, of male and female individuals respectively, where the large karyosome is still connected by a fibril with one of the two granules. A distinguishing feature in all the cases I have observed is that in the female nucleus the paired granules are radially arranged, while in the male they are tangentially arranged. The condition seen in the female individual of fig. 11, where the inner of the two granules has undergone a further unequal division, a still smaller granule remaining connected with it by a distinct centrodesmose, apparently represents a later phase which I have not seen in a male gametocyte. An important question is: Are these paired granules to be regarded as constituting kinetic elements (centrosomes) solely, or as representing small karyosomatic elements (i.e. containing also chromatin)? That they contain a division-centre does not require to be emphasised, as this fact is clear from the various centrodesmoses I have described and figured in connection with them, in both male and female nuclei. In my opinion it may be regarded as certain that the very small peripheral granule seen, for instance, in fig. 11 is a centrosome (or centriole), still in connection with its fellow one; as, however, the body at the other end of the fibril is slightly larger, it may be, perhaps, that this latter

element is really a very small daughter-karyosome, possessing a certain amount of chromatin which encloses the true centriole (cf. the very small karyosome and centriole in Hepatozoon canis, see p. 203). If this be so, the other granule of the pair must also be interpreted as a small karyosomatic element, and, of course, also the corresponding pair of granules in the male nucleus.

To understand the exact significance of the somewhat complex system of divisions and resulting elements which I have described, a study of their behaviour during the further development, i.e. gamete-formation and fertilisation, would be necessary. From a consideration of figs. 14–16 it may perhaps be suggested that the more external of the paired granules, situated usually just outside the clear nuclear zone, represents a further elimination of unrequired nuclear material, possibly a kind of maturation-process; but I have no indication whether the same explanation holds good in the case of the male forms. Lastly, with regard to the large karyosome itself. Does this body contribute any of its store of chromatin to the general chromatic material during the growth of the gametocyte, or is it entirely eliminated as unnecessary? In this connection one point which I have noticed may be mentioned. The karyosome is slightly but distinctly larger in a male nucleus which does not show the chromatic zone than in one which possesses this feature (cf. figs. 18, 19, and 20, 22). This may possibly indicate, in the latter case, some augmentation or replenishment of the chromatin of the general nuclear substance and a corresponding diminution of the amount held by the karyosome.

It will be clear, I think, that in regard to the essential features the nuclear constitution of both male and female gametocytes of Leucocytozoon ziemanni shows a close agreement, and this notwithstanding the apparently pronounced differences shown when they are respectively stained by Giemsa. It is remarkable how constant in appearance, on the whole, the nuclear condition is found to be; and this fact adds, of course, to the difficulty of interpreting the elements

observed While, however, the male and female nuclei of Leucocytozoon are fundamentally similar in type, there is no possibility of mistaking the one for the other, even in films stained by iron-hæmatoxylin, on account of the constant differences in detail. As I have already mentioned, Berliner's figs. 50 and 53, Pl. 29, which he regards as representing male gametocytes, do not agree at all with the characteristic appearance I have found and above described. The nucleus itself is figured as round, instead of being, as it almost invariably is, a pronounced oval in shape; and although it is somewhat larger than that of the female individuals which Berliner figures, it is nothing like the size which the male nucleus usually is. Moreover, the central area is stained more deeply, like that of the female forms, instead of being pale, even paler than the surrounding cytoplasm, as in the male forms; and lastly, there is no sign of the peripheral chromatic zone. The associated, intensely staining body is also very large, like the karyosome of the female gametocytes, and there is no indication of the small paired elements close to it. In short, I feel almost certain that the individuals figured by Berliner as of male sex are really also female forms (cf. his fig. 50 and my fig. 13, for instance).

I have dealt first with the nuclear structure of Leucocytozoon for two reasons: firstly, because in spite of its somewhat complex character it is not nearly so difficult to make out satisfactorily, on account of the large size of the parasites and the absence of pigment-grains, as is that of Halteridium, when fixed by a wet method and stained with iron-hæmatoxylin; and secondly, because it is more readily comparable with the nuclear condition found in the young forms of "Adelea" and Karyolysus. I have now to consider the nucleus of Halteridium, and will again begin with the female gametocytes. Berliner (loc. cit.) in the explanation of his figures of this parasite says nothing at all about the sex; so far as his figs. 58–60, of fairly large or adult individuals, are concerned, these certainly represent female forms. No male forms are figured, just as I maintain is the case with his figures of Leucocytozoon. The appearance

of the female gametocytes, according to Berliner, also agrees on the whole with the condition I have found. In most individuals the nucleus has a close resemblance to the characteristic flagellate type of nucleus. It appears as a very clear, round area, of relatively small size, which is sharply marked off from the surrounding cytoplasm and is most probably limited by a definite membrane; in the centre is a prominent, intensely staining karyosome (figs. 24–27). Berliner figures distinct rays passing from this central karyosome to the periphery of the nucleus. I certainly believe in the presence of these rays, serving, as it were, to sling the karyosome in position, but I cannot figure them for the simple reason that, even under the best optical conditions at my disposal, I am unable to actually see them myself; and I may say that others, who have kindly scrutinised several individuals on my preparations with this object, have also failed to discern them. Nevertheless I remember perfectly well once showing one of these preparations to my colleague Miss Robertson, then working in this laboratory, and she distinctly saw some rays in two or three cases, and sketched them for me. Hence, in the determination of these extremely delicate and difficult points one's own powers of vision are an important factor. Very frequently, at one side of the nucleus and usually close to, almost in contact with the membrane is a distinct granule, which is small and does not stain black so intensely (figs. 24–27). Now and again an obvious fibril or spindle connects this granule to the karyosome in the nucleus (cf. also Berliner's figures).

This was the nuclear constitution of Halteridium as I knew it when I wrote the postscript (à propos of Berliner's figures) to the paper by Minchin and myself (**5**) on the comparison of the nuclear structure of Hæmogregarina triglæ and Trypanosoma raiæ, and when I wrote the note on Halteridium fringillæ in my first study on Avian Hæmoprotozoa (**9**). It will be generally admitted, I think, that in view of the pronounced difference shown between this type of nucleus and that of Hæmogregarines (as the

latter was then known), when both were stained by a reliable cytological method, I was at the time quite justified in regarding the nuclear condition in Halteridium as corresponding closely to the karyosomatic type of nucleus seen, for instance, in a Trypanosome; and, further, in considering the definite, small associated element to represent a kinetonucleus in a "rückgebildet" condition as Berliner suggested. As a matter of fact, even until quite recently, and since I have realised the essential Coccidian nature of the nucleus of Leucocytozoon, I have been at a loss to explain this apparent resemblance of the Halteridium-nucleus to the binucleate condition and its difference from that of Leucocytozoon.

It is only within the last few weeks that I have learnt the true explanation of the matter and at last definitely settled, as I consider, the meaning of the nuclear appearance seen in Halteridium. The mistake has really been, I believe, in comparing the small associated granule, seen in films stained by iron-hæmatoxylin, with the conspicuous, deeply staining organella seen in Giemsa-stained smears, at any rate so far as regards the adult parasites. It so happens that some of my best iron-hæmatoxylin preparations of H. noctuæ are from an owl which had a heavy infection, and in which the great majority of the parasites were young, or intermediate-sized forms, relatively few being full-grown individuals. Looking over these at the time they were made, and again before writing the postscript above alluded to, I remember noting the general uniformity which was apparently presented by the nuclear structure. The small forms, the intermediate-sized ones and the few large parasites I came across all showed the karyosomatic type of nucleus, with or without the small accessory granule (and this is to be regarded, of course, as the regular condition, cf. figs. 24–27). As I then remarked, what I observed corresponded closely with what Berliner had figured. This being so, I did not undertake any systematic searching of these preparations at that time, as I wanted to continue first my study of the Avian Trypano-

somes. I naturally concluded that both Berliner and I myself had seen the same nuclear condition as that which I had considered to represent nuclear dimorphism when found on Giemsa-stained smears. I remember putting aside these wet preparations of Halteridium until a convenient opportunity for their detailed study should come along, with the thought that there was at least one point which was extremely difficult to determine from an iron-hæmatoxylin preparation, namely whether a particular individual was of male or female sex; it appeared to me as if, notwithstanding the well-marked distinction between the male and female nucleus after staining with Giemsa, the nucleus of both kinds of gametocyte was really of essentially the same form and structure, and the same view seemed to have been taken by Berliner, since he did not distinguish the sex.

Having found, however, since I began to study the cytology of Leucocytozoon ziemanni, that there is a constant difference between the nucleus of the male and female gametocytes respectively when stained by iron-hæmatoxylin just as in the case when stained with Giemsa, it was necessary to return to the Halteridium and try and settle the question as regards that form. Fortunately, I have recently obtained another chaffinch with a fairly good infection of H. fringillæ, in which most of the parasites are approximating to the adult condition and whose sex can therefore be readily distinguished. This time I at once made some iron-hæmatoxylin preparations, the examination of which happily enlightened me upon the whole question, in quite as great a measure as the study of Giemsa-stained ones helped to lead me astray in the first place. With the knowledge thus gained, I turned once more to my preparations of H. noctuæ, and have now been able to ascertain that the nuclear structure here also shows the same constant differences in the male and female forms.

In figs. 28, 29 are seen male gametocytes of H. fringillæ, and in figs. 30, 31 the corresponding forms of H. noctuæ. Both the red blood-corpuscles and the adult individuals of

the species of Halteridium infecting them are distinctly larger, it will be noticed, in the case of the little owl than in the case of the chaffinch. Hence the cytological details can be made out with somewhat less difficulty in the gametocytes of H. noctuæ, though of course not nearly so readily as in Leucocytozoon. It happened very fortunately that in one of my infected owls, the Halteridial parasites possessed, for some reason or other, very little pigment; many of my figures are drawn from this series of preparations, because in such a case there is no possibility of confusing the nuclear elements with pigment grains.[1] As is apparent from the figures, the nuclear structure agrees closely with that of L. ziemanni, and therefore a detailed description is unnecessary. As regards the large, oval, pale nuclear area in the male forms, I have never observed any indication of the peripheral zone of deeply staining chromatic grains, which are often so prominent in L. ziemanni; whether this is because they are not developed in the male nucleus of Halteridium, or merely because I have not succeeded in getting them to stain, I cannot say. There is, however, the same small, spherical, peripherally situated karyosomatic body, which now and again can be distinctly seen to be surrounded by a clear halo (fig. 30); and, close to it, the same dumb-bell shaped or else double centrosomic element.[2]

Turning now to the female gametocytes, it was the observation of the large, adult parasite (H. fringillæ) drawn in fig. 32 which suggested to me the explanation of the difference generally to be seen between the female nucleus of Halteridium and that of Leucocytozoon. In the

[1] It is perhaps scarcely necessary to say that this rather unusual feature does not imply that the nuclear details themselves differ at all from the condition found in other cases, where the parasites have the usual supply of pigment grains; the nuclear structure is obviously quite similar in my figures of H. fringillæ, which show numerous grains.

[2] In the case of Halteridium, these granules are so minute that it is difficult to believe they can be anything but the actual centrioles themselves.

individual figured, the conspicuous karyosome no longer occupies a more or less central position within the nucleus, but has passed distinctly to the outside, and bears apparently the same relation to the general nuclear substance as does the karyosome of the female individuals of L. ziemanni drawn in figs. 13–16. The chief points of difference to be noted are that the nuclear substance is here so faintly stained that it appears more like a spherical space than a nucleus; and secondly that I cannot (in this particular instance) make out any centrosomic granule. A similar condition is seen in figs. 33–35 of H. noctuæ, but two of these parasites show a distinct centrosome which is apparently intra-nuclear, though it may, of course, be lying near the upper or lower surface. The nuclear condition in this case agrees very closely with that of the female gametocyte of L. ziemanni drawn in fig. 13. I have not observed a single instance, however, where there are two granules in connection with the female nucleus of Halteridium, such as I have described as of frequent occurrence in Leucocytozoon. It is most probable, I think, that the centrosome[1] seen in figs. 33, 34 is the same element as that situated at the limit of the nucleus in figs. 24–26, but I have not found it connected by a fibril to the karyosome, where the latter has passed to the outside of the nucleus; the fibril perhaps disappears when the karyosome changes its position.

There can be no doubt, I think, that the smaller, intensely staining nuclear body in H. fringillæ (as seen when the parasites are stained by Giemsa), which I originally regarded as representing a kinetonuclear element, corresponds, not to the small peripheral centrosomic body seen in iron-hæmatoxylin preparations, when the nucleus has the condition shown in figs. 24–26, but to the karyosome, when this has passed to the limit of, or outside the nucleus (as

[1] In the case of the female nucleus also, I think it is preferable to regard this single granule as a centrosome only. I have not observed any secondary divisions or any further elimination (?) of small karyosomatic portions, as in the female nucleus of Leucocytozoon.

in figs. 33–35); at all events so far as large or adult individuals are concerned. That this is really the case is borne out by a fact which I noticed several times, namely, that only a certain proportion of the larger female forms of Halteridium (more, I should say, in H. fringillæ, fewer in H. noctuæ) show this characteristic additional element, in Giemsa-stained preparations; whereas practically all the female individuals of Leucocytozoon ziemanni exhibit it. We arrive, therefore, at the important result that when the female nucleus of Leucocytozoon is compared with that of Halteridium in the same phase, the two are found to be of essentially the same type of structure. Their apparent dissimilarity, as frequently observed, is due to the fact that in Halteridium the karyosome retains its central position within the nucleus throughout the period of growth of the gametocyte, and does not pass to the outside until the parasite is full-grown. On the other hand, in Leucocytozoon the karyosome appears to be always at the edge of, or else outside the nucleus, even in young or intermediate-sized individuals; I have never seen it within the central nuclear mass. This expulsion of the karyosome, which doubtless represents here, as in other cases, an elimination of unrequired chromatic material or "nuclear purification," thus takes place very early in the development of the macrogametocyte of Leucocytozoon, but only at a comparatively late stage in that of Halteridium.

The facts I have observed and described above finally settle, in my opinion, the question of the connection of Halteridium noctuæ (and equally, of course, of Leucocytozoon ziemanni) with Trypanosoma noctuæ. It appears to me that these parasites have no direct connection whatever, either ontogenetic or phylogenetic. As readers of my first study on avian parasites (loc. cit.) will be aware, I felt then compelled to relinquish the view that Halteridium and Trypanosoma were phases of one life-cycle, though I still considered that Halteridium was to be derived from a Trypanosome-like parasite, which had become permanently

intra-cellular, in view of its possession (as was then thought) of the binucleate condition and of a typical Flagellate, karyosomatic type of nucleus. There may be some among those who uphold the locomotor or kinetic view of the karyosome who will even yet be inclined to say, Why should not the conspicuous, deeply staining body associated with the nucleus in Leucocytozoon and Halteridium still be regarded as representing a kinetonuclear element, perhaps in a "reduced" or non-functional condition?

The following are very strong reasons, I consider, against maintaining any longer the view that these parasites do exemplify the binucleate condition, as it is found, for example, in the case of a Trypanosome. In the first place, as I have shown in the preceding section (Note III of this series), the typical karyosome cannot be considered as a "locomotor component" at all; there is no evidence whatever that the karyosome itself stands in any special relation to the kinetic activities. Secondly, from the comparison of the true nuclear condition occurring in Leucocytozoon and Halteridium with that obtaining in the Hæmegregarine, Karyolysus lacertæ, and in certain phases of different Coccidia, it seems evident that the so-called kinetonuclear element in the first-named forms represents in reality the karyosome of these other parasites. Lastly, but by no means of least importance, when Halteridium and Leucocytozoon apparently show nuclear dimorphism, according to Giemsa-stained preparations, the nucleus itself is seen in films stained by iron-hæmatoxylin to be no longer of the well-known karyosomatic type, i.e. not comparable to the trophonucleus of a binucleate Flagellate; in short, as is clear from the study of my figures of Halteridium, the prominent extra-nuclear body is the karyosome of the nucleus.

The association of Halteridium and Leucocytozoon (and also, in all probability, of Proteosoma and the malarial parasites) along with the Hæmoflagellates in the group Binucleata has therefore to be given up. These Hæmosporidia, equally with the Hæmogregarines, must be regarded

as closely allied to the Coccidia; it seems to me now that there is no longer any reason for supposing that they are derived from a binucleate form, such as a Hæmoflagellate. It has been a great disappointment to me to find that the view so elaborately worked out by Schaudinn and apparently so firmly based on facts, which I in common with many other Protozoologists adopted enthusiastically, has had to be abandoned, step by step, until the entire edifice is seen to be without any true foundation whatever. From my own work I feel persuaded that the principal if not the only basis upon which Schaudinn built was that which I have above indicated, namely the remarkable resemblance between the nuclear condition of the female gametocytes of Halteridium and Leucocytozoon, when stained by the Romanowsky method, to that which a Trypanosome might be expected to show if in a resting phase. I greatly doubt, indeed, whether Schaudinn ever saw the nuclei of these gametocytes stained by iron-hæmatoxylin; certainly no figures of individuals so stained are given in the recent published collection of his works (7). From the study by Minchin and myself (4) of this question, more especially from the standpoint of the Trypanosomes, and also from the present study of the cytology of the intra-cellular parasites, it must be admitted that no real evidence of any kind can be found to support Schaudinn's view.

Addendum.

In view of the publication quite recently of a paper by Prowazek (5A), on the "Geschlechtsdimorphismus der Trypanosomen," I feel obliged to add a few remarks to this note. Prowazek still maintains Schaudinn's view that Leucocytozoon and Halteridium represent, in each case respectively, merely the sexual phases of a Trypanosome. He thinks that Mayer ('Arch. Protistenk.,' xxi, 1911), has sufficiently proved this idea in the case of Halteridium, and he himself endeavours to show that an actual connection

exists in the case of the Leucocytozoan and Trypanosome parasites of fowls (Sumatra). So far as regards Mayer's account of the development of Trypanosomes from Halteridia, I shall have to criticise this in a later memoir; here I must confine myself to a brief consideration of the above-mentioned paper by Prowazek.

In the first place, it is impossible not to comment upon the appearance presented by the parasites in the figures on Prowazek's plates. I have pointed out above how frequently the figures hitherto given of Leucocytozoon have represented poorly fixed or stained specimens; but I do not recollect ever having seen any which are quite as bad as some of those on the plates in question. Speaking for myself, it is no exaggeration to say that, from many of the figures, taken by themselves, it is impossible to tell what they are meant to represent, so dreadfully are the parasites distorted and disorganised. It is obvious that no conclusion or interpretation can be accepted which is based upon preparations such as those from which these figures are taken.

Minchin and Woodcock (**4**), in their paper published only a month or two before Prowazek's appeared, and which presumably that author had not seen, have fully discussed the subject of the possible connection of Leucocytozoon ziemanni and the trypanosome of the little owl—the very parasites, i. e., on which Schaudinn worked—but it is not out of place to repeat here the main conclusions at which we arrived. In spite of numerous and prolonged living examinations we never observed the least sign of the passage from one form into the other—in either direction; nor is there the slightest evidence to this effect in any of our permanent preparations. The better fixed and stained these are, the more closely the Leucocytozoon agrees in form with the appearance presented in the living condition; it is remarkably uniform, and scarcely varies at all.

I may mention here a point which I have not referred to in the preceding pages of this note with regard to the true

nature of the characteristic spindle-like prolongations invariably found in connection with the fully grown forms of L. ziemanni (and some other species). While I still held the view that this parasite (as also Halteridium) was a Binucleate and phylogenetically derivable from a Trypanosome-like form (9), I thought it most probable that, at all events in the proximal portion of these prolongations, there was some ectoplasmic layer belonging to the parasite which helped to produce the prolongations. In the case of species where the infected leucocytic host-cell remains rounded and does not develop any horn-like prolongations (as in L. fringillinarum), I regarded the ectoplasmic layer as having been completely lost. However, in my cytological study of L. ziemanni, the results of which have been given above, I have found nothing to support the presence of any ectoplasmic layer. In properly stained individuals (whether stained by Giemsa or by iron-hæmatoxylin) there is no real distinction or differentiation to be made out between the most proximal region of these prolongations, i. e. nearest to the more deeply staining cytoplasm of the parasite, and the distal portions towards the tip. In the great majority of cases the staining (which is always pale) is quite uniform in tint, only becoming gradually fainter as the prolongation narrows to its extremity (figs. 5–7, 10–12, 18, 22). Very occasionally, in Giemsa-stained smears, a space-like area can be seen, which is probably more or less artificial. In short, these prolongations undoubtedly represent solely the altered and extended cytoplasm of the infected leucocyte, this characteristic change being caused by the stimulus of the invading parasite as it grows. I gather that Wenyon (8) was inclined to this view in his account of L. neavei. An additional reason in favour of it is supplied by the facts in regard to the nuclear structure which I have made known, which indicate the essentially Coccidian nature of Leucocytozoon, since the Coccidia lack, of course, any differentiated ectoplasmic layer.

To refer now to certain other points raised by Prowazek,

this worker considers that he obtained evidence which pointed to a Trypanosome enveloping a red blood-cell and proceeding to "take up" its nucleus. As we emphasized in our paper, we searched in vain, time after time, for signs of such a metamorphosis; the utmost we found to occur was the attachment merely of a Trypanosome to an erythroblast or a uninuclear leucocyte by one extremity, which might be either the flagellar or the aflagellar one. Two features very difficult to explain on the assumption that a Trypanosome thus passes into or becomes a Leucocytozoon are (A) the fact that the latter parasite, in its well-known form, shows always male and female individuals, whereas a similar distinction cannot be made out in the case of the Trypanosomes; and (B) the fact that quite small intra-cellular Leucocytozoan parasites occur, which certainly grow up into the characteristic adults, since intermediate-sized forms can be found. With regard to the remarkable phenomenon described by Prowazek of a parasite (a so-called "agamont") becoming separated from its original host-cell, but taking a part of the nucleus and the cytoplasm of the latter with it and penetrating with these elements into a fresh host-cell, I can only say that in all my experience I have never seen anything which could in the remotest degree suggest such an occurrence. Prowazek's figs. 2–5, pl. i, are supposed to show different stages in this process; but I cannot gather anything of the sort from them. Lastly, Prowazek also considers that the gametocytes of Leucocytozoon undergo a division, usually into two, which is considered to be longitudinal and thus to indicate the Trypanosome-character of these forms. The author says that both male and female forms may so divide, but adds the very significant remark that in some cases the two resulting individuals are not of the same sex, but one is male and the other female. From Prowazek's figures 8–17, it is perfectly clear that we have really to do here with the same condition which I have described in Halteridium (**10**), and which had been previously found in Hæmocystidium. As I discussed in that note, there can be little or

no doubt that it is a question of a double infection of the host-cell, i.e. by two small individuals which may be of the same or of opposite sex, which grow up in contact with each other. And I am practically certain that the same explanation holds good also in the present case of Leucocytozoon.

In conclusion, I am sorry to say that, in my opinion, Prowazek does not bring forward a particle of reliable evidence which is of any use towards rehabilitating Schaudinn's unfortunate view.

The Lister Institute;
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EXPLANATION OF PLATES 9 AND 10.

Illustrating Dr. H. M. Woodcock's "Notes on Sporozoa. II–IV."

PLATE 9.

[All the drawings are magnified 2000 times linear. All the figures, with the exception of figs. 1, 23, 41–43, relate to Karyolysus lacertæ (Danil.). Figs. 1–18 are from preparations stained with Giemsa].

Fig. 1.—Uninfected red blood-corpuscle.

Figs. 2–8.—Young parasites, with nucleus in the earlier phase, near the middle of the body.

Fig. 3.—A free individual, which has not yet penetrated a blood-corpuscle.

Figs. 9–18.—Older forms, with the nucleus in the later phase, situated (except in fig. 14) near one end of the body.

Figs. 14 and 15.—U-shaped forms.

Figs. 16–18.—Stout, bean-like individuals, resulting from the fusion of the two arms of the U. In figs. 17 and 18 the cytoplasm of the host-cell is not visible, and the host-cell nucleus forms a "cap" round one or both ends of the parasite.

[Figs. 19–43 are from wet films, stained with iron-hæmatoxylin.]

Fig. 23.—Uninfected red blood-corpuscle.

Figs. 19–22, 24–29.—Younger parasites, showing the karyosome (or else two karyosomes) closely associated with the nucleus.

Fig. 20.—Individual showing the promitotic division of the karyosome, the two halves being still connected by a spindle.

Fig. 21.—Small, free individual.

Figs. 30–40.—Older individuals, with the nucleus near one end of the body. In figs. 31, 37, a karyosome is still associated with the nucleus.

Figs. 32–36.—Parasites showing different stages in the alteration and gradual disappearance of the unused karyosomatic material.

Figs. 30, 39 and 40.—Individuals showing no sign of the karyosome or its remains.

Figs. 37 and 38.—Individuals surrounded by a distinct envelope, and in connection with which nothing whatever can be seen of the cytoplasm of the host-cell.

Figs. 41–43.—Merozoites or very young schizonts of "Adelea ovata" (Barrouxia alpina, according to Schellack and Reichenow).

Fig. 41.—A single large karyosome is present.

Fig. 42.—The karyosome has divided into two by a promitotic division, the connecting fibril being still present.

Fig. 43.—Four karyosomes of unequal size are present, resulting from further division.

PLATE 10.

[All the drawings are magnified 2000 times linear. I am indebted to Miss Rhodes for kindly drawing figs. 4 and 7. Figs. 4–10 are from Giemsa-stained smears; all the others from iron-hæmatoxylin stained films.]

Figs. 1–3.—Merozoites or very young schizonts of "Adelea ovata"

(Barrouxia alpina), showing two or more karyosomes in connection with the nucleus.

Figs. 4–6, 8.—Female gametocytes of Leucocytozoon ziemanni. (The parasite of fig. 4 is slightly flattened out.) *c.* General cytoplasm of parasite, containing its nucleus, and the associated karyosome. *h.c.* Cytoplasm of host-cell (leucocyte), prolonged into two tails or horns. *n.* Nucleus of host cell, elongated and dumb-bell shaped.

Figs. 7, 9 and 10.—Male gametocytes of L. ziemanni. Fig. 7 shows the general appearance of the nucleus, figs. 9 and 10 a much less common appearance. (The parasite of fig. 7 is slightly flattened out.) Lettering as in fig. 4.

Figs. 11–17.—Female gametocytes of L. ziemanni, showing details of nuclear structure. (To save space, in many cases only the middle portion of the parasite and of the elongated host-cell nucleus are shown.)

Figs. 18–23.—Male individuals of L. ziemanni, to show the details of nuclear structure. (In some of these figures also the spindle-like prolongations of the host-cell are omitted.)

Figs. 24–26.—Small, intermediate-sized and fairly large female individuals of Halteridium noctuæ, to show the nuclear condition as generally seen.

Fig. 27.—Large female form of H. fringillæ; similar nuclear condition. (Note the much smaller size of both blood-corpuscle and parasite in this case.)

Figs. 28 and 29.—Large and fairly large male gametocytes of H. fringillæ, to show the nuclear condition.

Figs. 30 and 31.—Ditto of H. noctuæ.

Fig. 32.—Large adult female individual of H. fringillæ, to show the extra-nuclear karyosome corresponding to the usual condition seen in L. ziemanni.

Figs. 33–35.—Large or fairly large female forms of H. noctuæ showing a similar or almost similar nuclear condition.